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Editorial report

In 1989, the previous *New Zealand Journal of Experimental Agriculture* was renamed and reshaped into the *New Zealand Journal of Crop and Horticultural Science* with the volume numbers continuing.

1989 was a very successful first year for the *New Zealand Journal of Crop and Horticultural Science*. Volume 17 comprised 375 pages with 43 papers, 7 short communications, 2 plant disease records, 1 technical note, and 6 book reviews.

The number of submissions was slightly lower than in previous years; this was expected following the change in scope of the journal. 56 papers were submitted in 1989, with 8 short communications, 2 plant disease records, 1 cultivar release, and 8 book reviews. Of these, some have already appeared in Volume 17, some will appear in 1990, in Volume 18, and 25% have been rejected. The most common reason for rejection has been poor experimental design, followed by poor presentation. Contributors are reminded to read the Information for Authors which is published in the first issue of each volume.

Processing times have been reduced for Volume 17; average time from submission to acceptance was less than 5 months, and average time from acceptance to publication was 3 months.

At the last meeting of the joint Editorial Advisory Board for the *New Zealand Journal of Crop and Horticultural Science* and *New Zealand Journal of Agricultural Research*, it was decided that a joint Board was no longer appropriate, as the new scopes of the two journals no longer overlapped. *New Zealand Journal of Crop and Horticultural Science* therefore enters 1990 with a new Board chaired by Dr R. Bieleski. I thank those members of the joint Board who are no longer on the new Board for their help over the last year.

This month I leave DSIR Publishing to travel overseas. I have been Editor of this journal for nearly 2 1/2 years and in that time I have implemented the change from *New Zealand Journal of Experimental Agriculture* to *New Zealand Journal of Crop and Horticultural Science*. Sandra Stanislawek and Gill Sutherland (co-editors of *New Zealand Journal of Agricultural Research*) will manage this journal until a new editor is appointed.

I encourage you all to support the editors so that the journal will go from strength to strength.

Endorsed by R. Bieleski
Board Chairman
New Zealand Journal of Crop and Horticultural Science

Sue Jolly
Editor, NZJCHS
February 1990

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We thank the referees listed below, and those who chose not to have their names listed, for their advice on papers reviewed during 1989.

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Papers published or under consideration elsewhere will not be accepted; but publication elsewhere of an abstract or extended summary does not preclude publication in full in this journal. In this context, restricted circulation of reports or theses does not constitute publication.

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Details of techniques and results which are not essential to the published paper but may be of interest to other workers should not be included. This material could be made available from authors on request, and this should be mentioned in the text.

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The International System of Units (SI), as described in NZS 6501: 1982, *Units of Measurement* (Standards Association of New Zealand), should be used wherever possible. Where non-SI units are used, the SI equivalents should also be given. In conversions, the number of significant figures quoted should truly represent the accuracy of the measurements or calculations made.

Papers should conform to internationally recognised codes of nomenclature (e.g., the *International Code of Zoological Nomenclature*). Generic and specific names should be italicised.

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These should be listed clearly and succinctly. It is important that any statistical technique employed be fully explained (if new) or referenced.

RESULTS

Here you present your own information and figures without reference to or discussion of other work. Tabular material and figures are especially important for providing comparative results without resorting to detailed textual descriptions. The tables and figures with their captions should be understandable on their own, and not rely on the supporting text.

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The results of your study are here related to those of previous studies. The results should be interpreted with the support of evidence or suitable references. Anomalous or unexpected results should be explained. Any conclusions offered should be listed clearly at the end of the Discussion.

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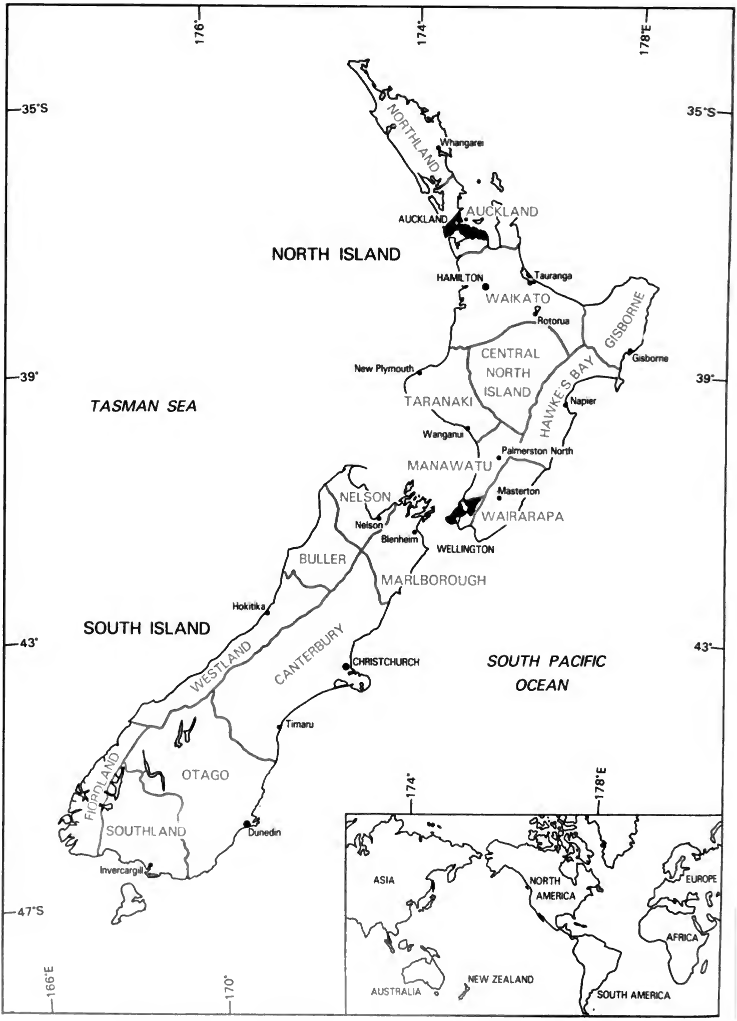
(Revised March 1990)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Flavonoid complement of cultivars of *Actinidia deliciosa* var. *deliciosa*, kiwifruit

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Abstract The flavonoid complement of *Actinidia deliciosa* var. *deliciosa* 'Hayward' leaf material is presented. In general, quantitative rather than qualitative variation was found between the flavonoid complements of the various cultivars of *A. deliciosa*. However, staminate cultivars contained an extra flavonol glycoside, quercetin 3-0-arabinoside, which was absent in the pistillate cultivars. This method of distinguishing the sexes was shown to be 80% reliable in the cultivars examined in the study. Juvenile leaf material was examined and it was shown that age was not a factor in flavonoid production in *Actinidia*. The fruit gave a similar flavonoid pattern to that of the leaf but the flavonoid levels were much lower.

Keywords *Actinidia deliciosa* var. *deliciosa*; kiwifruit; flavonoids; leaf; fruit; cultivars; staminate; pistillate; juvenile

INTRODUCTION

Actinidia deliciosa (A Chev.) C F Liang et A R Ferguson var. *deliciosa* 'Hayward' is essentially the only pistillate kiwifruit cultivar presently grown commercially in New Zealand. Only 'Hayward' fruit are currently exported. An industry based on a monoculture faces various problems such as the danger of susceptibility to particular pests and diseases, and the need to harvest and pack the whole crop within a limited period. DSIR Fruit and Trees has initiated a breeding programme on *Actinidia* to diversify its genetic base. There are many ways in which 'Hayward' could be improved and there is a desire to introduce commercially exploitable novelties. The present study of the flavonoid complement of *A. deliciosa* var. *deliciosa* is part of a larger project investigating the chemotaxonomy of *Actinidia*. This is being carried out in conjunction with DSIR Fruit and Trees and is aimed at increasing taxonomic knowledge about this and related species for possible use in breeding programmes.

Flavonoids are valuable markers in chemotaxonomy since they have been shown to give reliable species-specific patterns (e.g., Markham et al. 1985). With the exception of condensed tannins (Michaud & Ane-Margail 1977; Foo & Porter 1981) no previous studies of the flavonoids of *A. deliciosa* are available. A study of the chemistry of kiwifruit is reported by Luh & Zhang Wang (1984) but this does not include flavonoid components.

In this paper, the flavonoid pattern of several cultivars of *A. deliciosa* var. *deliciosa* foliage is presented together with the results of similar studies of the fruit, and a potential method of sex determination. The latter was undertaken to determine whether or not flavonoids could be used to determine the sex of the seedlings since *A. deliciosa* is dioecious and it normally takes 3–4 years for flowering to occur, thus delaying the breeding programme. The sex determination project involved checking juvenile leaf material because variations in the flavonoid profile with the age have been observed in other plants (e.g., Bohm 1987).

MATERIALS AND METHODS

Plant material used was as follows.

1. 1904 introduction *A. deliciosa* var. *deliciosa*, all grown at DSIR research orchard, Te Puke.
   Pistillate cultivars: 'Abbott'; 'Allison'; 'Bruno'; 'Elmwood'; 'Gracie'; 'Greensil' ('Constricted'); 'Hayward'; 'Jones'; 'Monty'.
   Staminate cultivars: 'Matua'; M51; M52; M56; 'Tomuri'.
   Inconstant males: M111; M117.
   Juvenile leaves of self-fruiting males and 'Hayward' vines.

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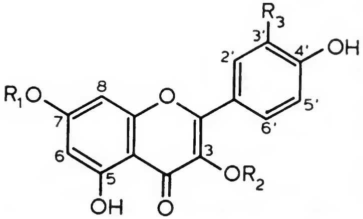

Fig. 1 Flavonol structures; **Kaempferol 3-0-glycosides** R1 = H, R2 = sugar, R3 = H; **Kaempferol 7-0-glycosides** R1 = sugar, R2 = H, R3 = H; **Quercetin 3-0-glycosides** R1 = H, R2 = sugar, R3 = OH; **Quercetin 7-0-glycosides** R1 = sugar, R2 = H, R3 = OH.

2. Accession from China, CCH1, *A. deliciosa* var. *deliciosa* now grown at DSIR research orchard, Te Puke.
3. Fruit of 'Hayward'.

Leaf material analysis

Large-scale flavonoid isolation from A. deliciosa *cv. Hayward*

One hundred grams dry weight leaf material (oven-dried 100°C) was ground in a Waring blender and extracted overnight at room temperature with EtOH:H_2O(1:1). The extract was dried and then applied in water to a polyamide column (Machery-Nagel CC6) and eluted with water to remove various non-flavonoid components. EtOH (95%) was then used to elute the flavonoids. Further purification was by paper chromatography: one-dimensional chromatograms (1D-PCs) were run on Whatman 3 MM chromatography paper using TBA(t-butanol:HOAc:H_2O 3:1:1) or 15% HOAc (acetic acid); the eluted bands were then cleaned up by two-dimensional (2D-PC) chromatography (TBA, 15% HOAc). Pure compounds were eluted from 2D-PCs and analysed by the various techniques outlined below.

Flavonoid isolation from other leaf samples

Ten grams dry weight (7–10 leaves) material was extracted as above. 2D-PC patterns were compared both with each other and with that for 'Hayward'. In some instances, e.g., CCH1, several 2D-PCs were run, the spots eluted, and further analyses performed to ensure that the plants were identical.

Sex determination

1D-PCs were run on plant extract in 15% HOAc. The lower half of the sheet was eluted and spotted onto a 2D-PC which was rerun in 15% HOAc after its initial TBA/15% HOAc development. This gave separation of the compounds which were then isolated and identified.

Analysis of the fruit

The fruit (1 kg sample) was peeled, then ground and extracted as above. The eluate was dried and applied in water to a water-washed amberlite XAD column to remove polysaccharide contaminants. After washing with water the flavonoids were then eluted with 95% EtOH. Further purification was achieved by applying the eluate in water to a polyamide column. Elution with water removed the remaining contaminants and the flavonoids were eluted with EtOH. Final clean-up was by 2D-PC.

Flavonoid identification techniques

Markham (1982) describes the techniques used in flavonoid analyses. Brief details of techniques used in this study are as follows.

Thin-layer chromatography (tlc): cellulose plates (F1440, Schleicher and Schull, 20 × 20 cm); solvents: HOAc 15% and 50%, TBA, benzene:HOAc:H_2O (125:72:5), n-butanol: EtOH:H_2O (4:1:2.2).

Ultraviolet light (UV, 366 nm) for viewing flavonoids on tlc/pc.

NA spray reagent (Naturstoff reagenz-A Roth, i.e., diphenylboric acid ethanolamine complex, Koch Light, 1% solution in MeOH) used for spraying tlcs and some 2D-PCs. NH_3 vapour for enhancing flavonoid spots on tlc and pc, both before and after spraying with NA.

Acid hydrolysis of flavonol glycosides: 3*M* HCl:MeOH (1:1), 100°C, 10 min.

Sugars: PC analysis nBuOH:benzene:pyridine: H_2O(5:1:3:3). Spots detected by a *p*-anisidine hydrochloride spray reagent.

RESULTS AND DISCUSSION

A. deliciosa contained several flavonols (Fig. 1) but contamination by tannins and fluorescent material proved a problem in the isolation of the pure flavonoids. It was found possible to remove most of these compounds using polyamide column

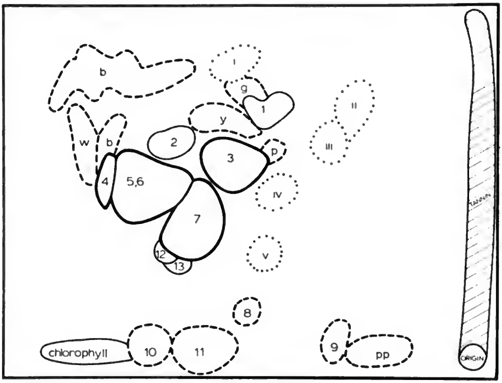

Fig. 2 2D-PC pattern of lavonoids in *Actinidia deliciosa* ar. *deliciosa* cv. Hayward. Representation of spots as seen under ultraviolet light (366 nm): hick outline = dark spot, major component; thin outline = dark spot, minor component; dashed outline = fluorescent spot, non-flavonoid unless numbered; dotted outline i–v) = trace flavonoids, not fully dentified, visible with NA. Numbering and lettering: all lavonoids are numbered; structures re listed in Table 2. Lettering refers o the colour of fluorescent spots: b = blue; w = white; g = green; p = pink; pp = purple; y = yellow. 1st un horizontal axis TBA, 2nd run vertical axis, 15% HOAc.

Table 1 Structures of flavonoids from 'Hayward' kiwifruit leaves and fruit.

Compound number	Flavonoid structure	Leaf[a]	Fruit[b]	Spot colour[c] UV	NH$_3$	NA
1	Quercetin 3-0-rhamnoside 7-0-glucoside	*		dark	olive	orange
2	Kaempferol 3-0-rutinoside	*	+	dark	olive	green
3	Quercetin 3-0-rutinoside	**	+	dark	olive	orange
4	Kaempferol 3-0-rhamnoside	**	+	dark	olive	green
5	Kaempferol 3-0-glucoside	*	+	dark	olive	green
6	Quercetin 3-0-rhamnoside	**	+	dark	olive	orange
7	Quercetin 3-0-glucoside	**	+	dark	olive	orange
8	Kaempferol 7-0-glucoside	*		yellow	yellow	yellow-green
9	Quercetin 7-0-glucoside	*		yellow	yellow	yellow-orange
10	Kaempferol	*		yellow	yellow	green
11	Quercetin	*		yellow	yellow	orange
12	Quercetin 3-0-xyloside	*		dark	olive	orange
13	Quercetin 3-0-arabinoside	*		dark	olive	orange

[a] Relative intensities using UV, ** strong, * weak.

[b] + indicates compounds present.

[c] Spot colours: UV = ultraviolet light (366 nm); NH$_3$ = ammonia vapour; NA = Naturstoffreagenz A (Roth) spray reagent.

chromatography. Although the non-flavonoid components, especially the fluorescent compounds, have not been identified, their presence may be of use in identifying the presence of *A. deliciosa*, e.g., in hybrid studies.

Flavonoid chemistry of 'Hayward' leaf material

The 2D-PC pattern of flavonoids of *A. deliciosa* 'Hayward' is illustrated in Fig. 2 with the exception of Compound 13, which has been included to illustrate its relative position compared with the other compounds, but is not present in 'Hayward'. As discussed below, Compound 13 is present in staminate cultivars. The identity of the compounds, along with their relative intensities and colour characteristics, are given in Table 1. The dominant flavonols are quercetin 3-0-rhamnoside (Compound 6), quercetin 3-0-glucoside (Compound 7), kaempferol 3-0-rhamnoside (Compound 4), and quercetin 3-0-rhamnoglucoside (Compound 3) with quercetin 3-0-rhamnoside being the most dominant. ^{1}H-nmr was used to identify Compound 3 as the 3-0-rutinoside (Mabry et al. 1970). By analogy, the kaempferol glycoside (Compound 2) is also assumed to be in the rutinose form. Flavonoids (i) to (v) (Fig. 2) are present at low levels. Whilst their presence is seen from an NA sprayed (100 mg ≡ dw) 2D-PC, it was not possible to fully identify these compounds. However, (ii) and (iii) are thought to be quercetin 3,7 diglycosides.

Sex differences

From an initial survey, it appeared that staminate and pistillate plants were distinguishable on the basis of their flavonoid complements. Examination of the cultivars listed in this paper showed that, in the pistillate cultivars, only quercetin 3-0-xyloside (Compound 12) is present, whereas the staminate cultivars also contain quercetin 3-0-arabinopyranoside (Compound 13). These compounds are present in small amounts and in some cultivars are hard to detect. However, about 20% of the cultivars did not conform to the above pattern. 'Monty' and 'Greensil' (pistillate cultivars) contained Compound 13 whereas 'Tomuri' (staminate cultivar) contained only Compound 12. This limits the usefulness of these compounds for identification in horticulture, but it is still of interest that such differences have been found.

The other group of plants considered in this part of the study were inconstant males, e.g., M111 and M117. These are plants which have bisexual flowers interspersed with staminate flowers; the bisexual flowers can produce fruit but usually they are small. M111 and M117 both gave staminate flavonoid patterns consistent with the idea that they are in fact fruiting males, but they cannot be distinguished from staminate cultivars using flavonoid chemistry.

Cultivars of *A. deliciosa*

A comparison of both unsprayed and sprayed (NA) 2D-PCs showed no qualitative differences between the various cultivars other than the sex differences already described. Quantitative differences do exist, both in flavonoids and in the amounts of chlorophyll and fluorescence present but were found to be unreliable for distinguishing cultivars of *A. deliciosa*.

A different accession of *A. deliciosa*, CCH1, gave the same flavonoid pattern as that from 'Hayward'; thus the pattern presented in this paper is considered to be a reliable flavonoid pattern for *A. deliciosa* var. *deliciosa*. This is important since all the other plant samples examined in this study originate from the 1904 introduction and thus would be expected to be very similar.

Juvenile leaf material

2D-PC patterns from leaf material of juvenile self-fruiting males and juvenile 'Hayward' (pistillate) were the same as those from the adult material. Male and female patterns, although very weak, could be seen after the rerun (15% HOAc) of the 2D-PCs (see sex determination methods). Thus, juvenile leaf material could be used to predict the mature plant pattern.

Fruit

The fruit of 'Hayward' contained very low levels of flavonoids compared with the leaf material but those identified were also present in the leaves. Identification was by co-chromatography (tlc). Relative concentrations of flavonoid (Table 1) are not listed because high levels of contaminants resulted in difficulties in obtaining a 2D-PC pattern.

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Dynamics of soil and plant nitrogen in cultivated and no-till spring wheat systems following old pasture

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Abstract When old pasture is cultivated, nitrogen (N) may be released in quantities that exceed crop demand. No-till cropping is an option that may be more conservative of N. This report considers uptake and distribution of N by a wheat crop established by full cultivation or no-till methods, out of 12-year-old clover/grass pasture. N uptake was more rapid in cultivated soil and in both systems was essentially complete after 40 days. The no-till treatment did show some further N accumulation later in the season during grain fill. Mineral N values and plant uptake rates indicate that N mineralisation was rapid in both systems but only just adequate in no-till. Sampling the soil to 1.5 m depth in the winter following the crop showed both systems to have similar and substantial (about 160 kg N/ha) quantities of NO_3-N moving through the profile.

Keywords nitrogen; wheat; pasture; mineralisation; cultivation; no-till systems

INTRODUCTION

Old grassland, natural or managed, is generally characterised by large quantities of nitrogen (N) in the supporting topsoil. When the sod is broken, much of this N may be mineralised and become available in excess of crop requirement. With the passage of time, the decline in soil N under conventional tillage is substantial and N availability may ultimately limit productivity (Sears et al. 1965; Carran 1983).

Systems involving minimum soil disturbance in crop culture (variously known as minimum tillage, conservation tillage, or direct-drilling) are designed to reduce structural damage to soils and soil erosion but may also generate a lower rate of N mineralisation. The relationship between tillage systems, in terms of N release, is by no means constant however. Rice et al. (1987) report that it interacts strongly with drainage class so that poorly drained soils release more N under no-till than conventional tillage. McMahon & Thomas (1976) showed that tillage systems also affected the potential for leaching with no-till systems giving lower mineralisation and higher leaching losses than conventional tillage. Lamb et al. (1985) present N budgets for several systems which indicate the loss of 16% of total N over 12 years of ploughing, compared with about 2% in no-till, wheat-fallow cropping. Deep leaching of NO_3-N was the largest component of the N budget that differed among their systems.

The experiment described here investigates the N utilisation by crops in cultivated and no-till systems and the accumulation of NO_3-N and NH_4-N in soil during the first crop following a pasture phase of more than 12 years.

METHODS

Within an area developed from old (> 12 years) pasture, adjacent areas were sown in wheat by either no-till or full cultivation. These areas were to be continuously cropped in cereals and had dimensions 100 × 10 m. An area of 20 × 2 m was marked in the centre of each and was used for all measurements. Wheat, cv. Otane, was sown on 30 September 1986. The cultivated treatment was ploughed 4 weeks before sowing and cultivated following local practice during that period. Existing vegetation was killed with glyphosate at sowing in the no-till area. Neither system had significant weed populations during growth of the crop. The soil was Kairanga silt loam with 0.40% N, 3.7% C, and pH 6.1.

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At regular intervals, four known areas, determined at random within the measurement area but separated by at least 1 m of undisturbed crop from any other concurrent or previous measurement area, were harvested by hand-pulling plants. From within each of these measurement areas, four 25 mm diam. soil cores were taken and separated into sections of 0–75, 75–150, and 150–300 mm depth. The plough layer was at 150 mm in the cultivated treatment. Comparisons between sampling times within tillage systems, and comparisons between the tillage systems at any sampling time were evaluated by two-tailed *t*-testing. Table 1 shows final yields of whole ears from plot harvests and grain yields (ex combine) from the larger areas that the plot were located in; also shown are the yields of the other cultivated and no-till areas located in the same paddock.

Plant samples from ear emergence to final harvest were separated into the components: leaves; stems; and ears. At the final harvest, ears were separated from the remainder of the plant. Roots were not measured. Plant parts were analysed for total P and total N (including NO_3-N).

Soil samples were sieved (2 mm) and extracted with 2*M* KCl (containing 5 µg/ml phenyl mercuric acetate) at a soil:extractant ratio of 1:10. This was competed within 3 h of sampling. The KCl extracts were analysed for NO_3-N and NH_4-N by automated colorimetry. Soil water content was determined gravimetrically on all soil samples.

RESULTS

Nitrogen uptake by crop

Fig. 1a shows the cumulative uptake of N by the wheat crops. Rapid uptake early in crop growth was obvious under both methods of tillage. Beyond 40 days after sowing (DAS) no significant uptake occurred. The principal component affecting difference in N uptake was plant yield, as % N remained very similar in all plant parts and sampling times (Fig. 1b). It should be noted that the residue fraction (all material except ears) had a significantly higher % N in the cultivated treatment (0.6% cf. 0.4%). The difference is small in absolute terms but has a large effect on C:N ratio of residues.

Table 1 Yields of hand-harvested plots and grain yields (ex combine) of the areas in which small plots were located and of adjacent cultivation or no-till plots. Values in parentheses are the standard error of the mean.

	Yield (t/ha)	
	Full cultivation	No-till
Hand-harvested (whole ears)	9.17 (±1.91)	7.62 (±0.94)
Yield ex combine	5.04 (±0.24) *n* = 3	4.59 (±0.48) *n* = 3
Yield of adjacent areas	5.53 (±0.39) *n* = 9	4.84 (±0.52) *n* = 6

P uptake by the crop

In contrast to N uptake, P uptake patterns (Fig. 1c) show a tendency to increase through to a harvest maximum. A clear advantage is seen in the cultivated system up to 90 DAS; thereafter the gap closes.

Soil mineral nitrogen

In a dynamic production-utilisation-loss system, point measurements of a property like soil mineral N need careful interpretation. The data presented in Fig. 2 showed that, despite the rapid uptake of N up to 40 DAS, both tillage systems were sustaining substantial quantities of N in the upper profile. In the no-till system, the 0–75 mm layer was most active during this time whereas high rates of mineralisation are apparent in the entire tilled layer of the cultivated system. The influence of the inverted surface layer can clearly be seen in the high values of the 75–150 mm layer in the cultivated system.

During the period 13 November 1986–7 January 1987 (43–98 DAS), no significant changes in the mineral N pools occurred nor did the systems differ until after rain fell on 17–23 January (49 mm), when mineralisation was obviously stimulated in the 0–75 mm layer of the no-till area and through to the plough layer under cultivation.

Soil sampling to 1.5 m in late June 1987 showed that accumulation of $NO_3^- + NH_4^+$ was similar in both tillage systems (Table 2). Calculation of a soil water balance indicated that soil water did not recharge to near field capacity until the month of sampling. The horizons sampled should, therefore, include all excess mineral N produced since cultivation less any gaseous losses.

Nitrification

A large proportion of the mineral N was NO_3-N at the earliest sampling, but the proportion of NO_3-N declined until 98 DAS. During the period of decreasing soil water availability, NH_4-N become the dominant form with little nitrification apparent until after the rain that fell before sampling at 127

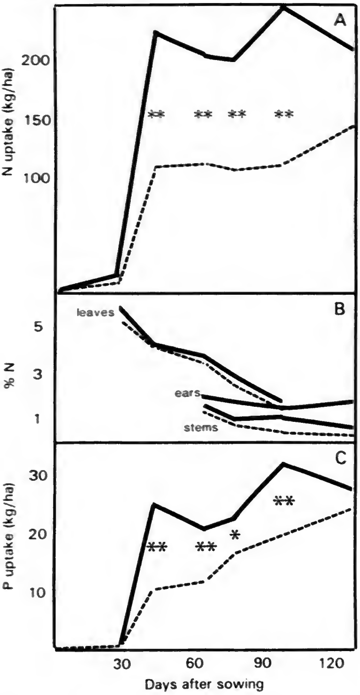

Fig. 1 Nutrient uptake in the two tillage systems throughout the growing season. A, N uptake; B, N content; C, P uptake. Solid lines = full cultivation, broken lines = no-till. In B, the lines for N content of ears cannot be separated graphically. * and ** indicate differences significant at the 0.05 and 0.01 levels of probability respectively.

DAS. All sampling depths showed an appreciable proportion of NO$_3$-N at times but the relative importance of in situ nitrification and NO$_3^-$ transport in the deepest layer is hard to judge.

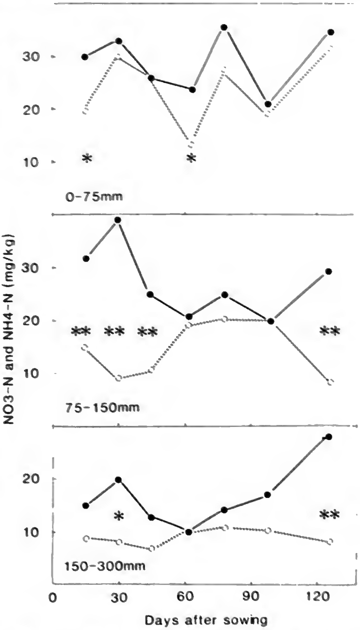

Fig. 2 Content of NH$_4$-N + NO$_3$-N in the three soil layers sampled. Solid lines = full cultivation, broken lines = no-till.

Soil water

Interpretation of the influence of soil water in the two systems is limited by having only gravimetric soil water content data. The two systems behave similarly with values remaining high through the first 30 DAS, declining (Fig. 3) until an early December rainfall, then drying progressively until the rainfall before the final harvest. The essential point is that soil water is unlikely to have been limiting crop growth before ear emergence in this season. The effects on N mineralisation were more distinctive however, as the period when NO$_3^-$ + NH$_4^+$ changed very little coincided with the driest rainfall period, and rewetting with a marked increase in mineral N content. Nitrification rates also appeared to have been influenced strongly by soil water availability with NO$_3$-N forming a less significant proportion of mineral N during the driest period.

Table 2 Midwinter soil sampling of NO_3-N + NH_4-N, to 1.5 m; June 1987. Values in parentheses are the standard error of the mean.

	NO_3^- + NH_4^+ (mg/kg)		
Depths (mm)	Cultivated	No-till	Estimated kg/ha
0–300	18.6 (± 2.9)	20.6 (± 4.6)	70
300–600	12.4 (± 1.8)	12.2 (± 6.0)	44
600–900	7.1 (± 2.4)	6.6 (± 3.4)	25
900–1200	4.6 (± 1.6)	3.9 (± 1.5)	14
1200–1500	3.1 (± 1.4)	3.3 (± 0.7)	10

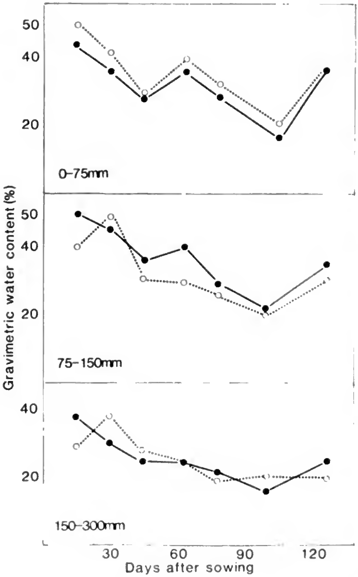

Fig. 3 Soil water content (% by weight) in the three soil layers sampled. Solid lines = full cultivation, broken lines = no-till.

DISCUSSION

With conventional tillage, plant growth was enhanced early in the season and nutrient uptake and yields determined by 44 DAS. The no-till treatment developed more slowly and ultimately attained a lower yield. The progressive increase in total P uptake from germination to harvest in the no-till treatment reflects this difference.

Both treatments appear to have mineralised enough N to meet crop demand as mineral N levels in the soil were still high at the end of the N uptake period. It is not possible to determine mineralisation rates from mineral N values but plant uptake and net change in mineral N to 300 indicate that, by 44 DAS, 227 and 117 kg/ha had mineralised in conventional and no-till respectively, and the bulk of that between 30 and 44 days. In terms of daily mineralisation rates, these are high values: 5.2 and 2.65 kg N/ha day using 44 days, and 16.2 and 8.4 kg N/ha using 14 days. Furthermore, this rapid net mineralisation does not continue past the period of rapid crop growth since there is no evidence for mineral N accumulation in the soil after that time. The continued uptake of P suggests that this is not related to soil water availability. Hart et al. (1979) report similar patterns of N uptake for wheat crops, and their comparisons with fallow soils suggest that the crop enhances mineralisation during the most rapid growth phase. Loss of N from the plant to the atmosphere (Quinn 1982; Harper et al. 1987) is a further factor that could give rise to a low estimate of N mineralisations.

This pattern of N uptake is different from those reported for winter wheat (Harper et al. 1987; Willson 1987) where total plant N increases progressively from stem elongation until maturity. Spring wheat examples are not readily available in New Zealand especially where N is available in quantity. Campbell et al. (1979), however, showed that N uptake continued until maturity under high rates of N and adequate water supply but where water became limiting, N uptake stopped and total plant N declined over time.

Mineral N contents of the soil in June showed a substantial quantity remained in both systems. The water balance calculated from September to June suggests that significant loss of NO_3-N by leaching was unlikely however. If the NO_3-N values are added to the crop uptake values, it can be seen that the full cultivation did result in a substantially greater release of N. However, the balances presented here are not complete and omit processes like immobilisation associated with straw decomposition which could require up to 50 kg N/t straw, as well as atmospheric losses. In addition, results may well vary in seasons of differing rainfall.

CONCLUSIONS

In the season studied, full cultivation mobilised a substantially larger quantity of N which was reflected in:

1. Higher product N, 202 cf. 146 kg/ha;
2. Higher residue % N, 0.6 cf. 0.4 % N;
3. Higher N availability for the stubble crop; c. 90 cf. 65 kg N/ha.

However there was no evidence that more N was available to loss processes at midwinter. The data collected do not allow assessment of losses to the atmosphere or differences that may have accrued in the organic fractions. Losses between cultivation and sowing have not been measured either but any leaching of NO_3^- that occurred during that time should have appeared in sampling to 1.5 m in June 1987.

Although the cultivated system clearly mineralised more N, the increased activity was confined largely to the rapid phase of crop growth. The no-till system conserved more N but was less productive as a consequence. The conservative effect did not extend much beyond 60 DAS and a large and similar amount of NO_3-N was present in both systems at midwinter. The potential for under-utilising the large amount of N released when pasture is converted to arable was not significantly affected by tillage method.

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Breeding and development of flue-cured tobacco cultivar 'Moskuaka 85'

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Abstract 'Moskuaka 85', a new cultivar of flue-cured tobacco, was developed for New Zealand growing conditions. It has resistance to three important diseases affecting tobacco grown in New Zealand: tobacco mosaic virus (TMV); black root rot; and verticillium wilt. 'Moskuaka 85' was derived from a cross made in 1973 between a TMV resistant Taiwanese flue-cured cultivar and a New Zealand breeding line resistant to black root rot and verticillium wilt. Resistance screening for the three diseases was performed from the F_2 to F_6 generations of the cross using standard pedigree breeding procedures. Twenty F_7 lines were evaluated for agronomic, chemical, and physical characters. Eight F_8 lines were entered in regional small-plot trials. After three seasons of evaluations at three sites, two F_{11} lines were tested in large-scale (2 ha) trials. 'Moskuaka 85' was released in 1985 as an F_{13} line.

Keywords flue-cured tobacco; disease resistance; tobacco mosaic virus; black root rot; verticillium wilt; genotype × environment interaction; plant breeding

INTRODUCTION

There are three major diseases of concern to the New Zealand flue-cured tobacco industry. They are tobacco mosaic virus (TMV), black root rot (*Trichocladium basicola* (Berkeley & Broom) Carmichael), and verticillium wilt (*Verticillium dahliae* Klebahn). All three diseases are widespread in the tobacco-growing district of Nelson, and have caused economic losses from time to time over the last 50 years. Within the genus *Nicotiana* there are good sources of resistance to TMV and *T. basicola.* These sources of resistance have been widely used in tobacco breeding programmes worldwide. TMV resistance is conditioned by a single dominant gene (N gene) which has been transferred into *N. tabacum* from the species *N. glutinosa* (Holmes 1938). This resistance is of a hypersensitive type and manifests as a necrotic local lesion when resistant plants are inoculated with the virus.

Black root rot is a soil-borne fungal disease and resistance is inherited polygenically (Clayton 1953). Sources of resistance have been obtained from within *N. tabacum* (Burk & Heggestad 1966). The above-ground symptom of black root rot is poor agronomic performance and the disease is identified by the presence of black lesions on the root system (Lucas 1975).

Since verticillium wilt was first identified in 1944 in New Zealand (Thomson & McLeod 1959) extensive research has been undertaken on methods of control (McLeod & Thomson 1959; Thomson & McLeod 1959; Wright 1968). No sources of resistance within other species of *Nicotiana* were found (McLeod & Thomson 1959). One of the principal areas of investigation since then has been to find host resistance within *N. tabacum.* As a result, several cultivars, mainly from the USA, with some degree of resistance to verticillium wilt have been identified (McLeod & Thomson 1959; Wright 1968). Some of these cultivars have been used extensively in the New Zealand verticillium wilt resistance breeding programme. Three New Zealand-bred cultivars with resistance to both verticillium wilt and black root rot have been released since 1966 (Beatson 1973; Wright 1973; Beatson et al. 1979). Genetic studies by Wright (1968) and Beatson (unpubl. data) on verticillium wilt resistance have shown that most of the genetic variation is controlled by additive factors. Plants infected with *V. dahliae* display the classical symptoms of vascular wilting. Tiger striping of leaves often occurs down one side of the plant (Thomson & McLeod 1959).

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In 1973, a cross was made at Riwaka Research Station between 'Vamfen Hicks', a Taiwanese TMV resistant flue-cured cultivar, and a New Zealand breeding line, 7278-202, with good resistance to both black root rot and verticillium wilt. Selection for resistance to the above three diseases was made in the segregating generations using the pedigree breeding procedure. Preliminary yield testing of F_7 lines was completed and eight F_8 lines were subsequently selected for regional farm trials. This paper discusses procedures used in the development of disease-resistant tobacco breeding lines and the regional trial series results with subsequent cultivar release.

MATERIALS AND METHODS

Breeding history

In 1973, a cross was made between a black root rot and verticillium wilt resistant breeding line, 7278-202, and 'Vamfen Hicks', a TMV resistant line (hereafter referred to as the 2072 family). The F_1 hybrid was grown in the glasshouse during winter 1973 to produce F_2 seed ready for field testing in the 1973/74 season. From the F_2 to F_6 generations, pedigree selection was practised (Allard 1960), based on resistance to the three diseases and plant type. Selections for agronomic performance were made on F_7 breeding lines. Selected F_8 lines were evaluated in replicated small-plot regional trials commencing in 1980/81 and evaluated for three growing seasons. As F_{11} lines, further selections were made for large-scale (2 ha) grower evaluations commencing 1983/84 season and continuing the following (1984/85) season. Bulked seed lots were used for the large-scale evaluations. In August 1985, a decision was made to release one of the F_{13} lines as a new cultivar.

Selection procedures

In the segregating F_2–F_6 generations, artificial inoculation was employed to screen for resistance to all three diseases. Black root rot resistance was assessed at the seedbed stage where sterilised beds were inoculated with oatmeal-sand cultures of *T. basicola* before seed sowing (Wright & Biss 1967). At transplanting, seedlings were lifted, their roots washed free of soil, and examined for resistance to black root rot by visual appraisal of their roots. Susceptible control cultivars were used to check the pathogenicity of *T. basicola*. Selected plants were then inoculated with a conidial suspension of *V. dahliae* (10^6 conidia/ml) by standing the plants in the inoculation solution for 1 h in bright sunlight (Hartill & Campbell 1976). The plants were then transplanted into a quarantine area where the natural infestation by *V. dahliae* was high. Susceptible control cultivars were used to check on infection levels in the field. Approximately 6 weeks after transplanting, each plant was inoculated with a TMV solution in the form of TMV-infected green leaves ground in a carborundum-based suspension (Gibbs & Harrison 1976).

The plants were treated in accordance with normal agronomic practices for flue-cured tobacco (Hawkes & Collins 1983) except that they did not have their inflorescences removed. This allowed subsequent seed saving from selected plants. Unopened inflorescences of the selected plants were bagged to ensure self-pollination (Wernsman & Matzinger 1980).

These procedures enabled selection for resistance to all three diseases on the same plants in one growing season; i.e., after flowering had commenced, plants which had been selected before transplanting for black root rot resistance, were selected simultaneously for verticillium wilt and TMV resistance. Selfed seed from resistant plants was thus obtained.

Replicated yield trials

A total of eight F_8 lines from the 2072 family were chosen for more extensive evaluation in regional farm trials. For comparison purposes, the standard commercial cultivars 'Kuaka 427' and 'Kuaka 860' were included in all trials. Three locations were chosen, to represent soil type and local climatic differences. The locations were Riwaka (Riwaka silt loam), Motueka (Hau stoney silt loam), and Motueka Valley (Sherry sandy loam). The lines were evaluated over three seasons (F_8 to F_{10} generation) at each of these sites. There were four replications per trial site with plot size being a single row of 25 plants. A randomised complete block design was used.

Measurements

Field measurements included days to flowering (from transplanting), number of harvested leaves, plant height (cm), and leaf length (cm) and leaf width (cm) of the fifth leaf from the top of the plant. Measurements were made on five random plants per plot. Days to flowering was calculated as 50% of plants per plot with at least one flower open.

Post-harvest measurements were as follows:

(i) net yield (kg/ha): of saleable leaf calculated from plot weights with adjustments for missing plants following the procedures of Crews & Jones (1962);

(ii) net grade index: weighted average desirability on a scale of 0–100 based on the international flue-cured tobacco grade system and adapted for use in New Zealand (R. W. James, Flue-cured grading desirability schedule 1966. DSIR internal report);

(iii) moisture at equilibrium (%): determined by placing a sample of cut tobacco (13 cuts/cm) in a conditioning chamber held at 25°C and 60% relative humidity for 72 h;

(iv) filling value (cc/g): by the standard piston and cylinder method (Artho et al. 1963) using cut, conditioned tobacco from (iii) above;

(v) reducing sugars (%); and

(vi) total nicotine alkaloids (TNA) (%) determined spectrophotometrically according to standard procedures (Harvey et al. 1969). Physical and chemical measurements were performed on composite plant samples.

Statistical analysis

Data were analysed using the following linear model:

$$H_{ijkl} = \mu + Y_l + S_k + YS_{kl} + R_{j(kl)} + G_i + GY_{il} + GS_{ik} + GSY_{ikl} + \varepsilon_{ijkl}$$

where:

H_{ijkl} = plot variable;
μ = population mean;
Y_l = year random effect where $l = 1$ to 3;
S_k = location random effect where $k = 1$ to 3;
$R_{j(kl)}$ = replication random effect where $j = 1$ to 4;
G_i = genotype fixed effect where $i = 1$ to 10;
YS_{kl} = years × location effect;
GY_{il} = genotype × years effect;
GS_{ik} = genotype × location effect;
GSY_{ikl} = genotype × location × year effect;
ε_{ijkl} = random error effect.

Expected mean squares for the mixed model used in the data analysis were determined (Steele & Torrie 1980) to allow appropriate *F*-ratios to be used in the hypothesis tests of interest. An approximate *F*-test was required for hypothesis testing of genotypes and therefore approximate degrees of freedom as proposed by Satterthwaite (1946) were used.

RESULTS AND DISCUSSION

Table 1 summarises the regional evaluation results. Yields of all breeding lines were at least equal to or better than the commercial cultivars 'Kuaka 427' and 'Kuaka 860'. For leaf quality, as judged by grade index values, most of the 2072 family breeding lines compared favourably with the two commercial cultivars. Breeding line 2072-85 was significantly better than the standard cultivars for filling value, a character which is important in tobacco manufacturing. There were significant differences among breeding lines for moisture at equilibrium, another important physical leaf character for manufacturing. TNA and reducing sugars were all within acceptable ranges in comparison with the two commercial cultivars. Plant height and number of leaves per plant of the breeding lines were similar to the standard cultivars while the 2072 family lines tended to flower slightly earlier than the standards. There was little difference in leaf length between the 2072 breeding lines. All of the breeding lines had broader leaves than the Kuaka cultivars. Coefficients of variation were acceptable for field trials (LeClerg et al. 1962).

Table 2 shows that genotype × environment interaction was non-significant for most characters, in agreement with other tobacco studies (Jones et al. 1960; Gupton et al. 1974). Genotype × location effect was significant for yield and plant height whereas genotype × year effect was significant for filling value, reducing sugars, plant height, number of leaves, and days to flowering. The second-order interaction component, genotype × location × year, was significant for leaf length only.

In Table 3, the F_1 hybrid between 'Kuaka 860' and 'Moskuaka 85' and the mid parent value is compared. There were no significant differences for characters between the F_1 and the mid parent value, apart from total nicotine alkaloids. Therefore, growing the TMV resistant F_1 hybrid offers no commercial advantage.

From the 2072 family regional trials, F_{11} lines 2072-85 and 2072-86 were advanced to large-scale (2 ha) farm trials. After two seasons and extensive evaluation of manufacturing properties, 2072-85 was selected for commercial release. In comparison with 'Kuaka 860' (the commercial cultivar of preference), 2072-85 was significantly better for filling value and had broader leaves. Line 2072-85 was released in 1985 as 'Moskuaka 85'. Currently, 'Moskuaka 85' represents 40% of the domestically grown flue-cured tobacco.

Table 1 Means of regional trials conducted over three locations and 3 years: agronomic, chemical, and physical characters.

Genotype	Yield (kg/ha)	Grade index	Filling value (cc/g)	Moisture at equilibrium (%)	Total nicotine alkaloids (%)	Reducing sugars (%)	Plant height (cm)	No. leaves (per plant)	Days to flower	Leaf length (cm)	Leaf width (cm)
Kuaka 427	2971	51.34	4.02	12.57	2.51	13.89	104.2	18.8	77.9	61.2	23.2
Kuaka 860	3011	51.43	4.09	12.90	2.43	15.32	110.8	18.0	75.1	62.0	23.2
2072-72	3046	51.48	4.25	12.66	2.52	14.48	105.1	18.1	73.7	60.9	24.1
2072-81	3268	50.56	4.04	12.90	2.46	15.69	99.2	18.9	74.7	60.3	23.6
2072-82	3358	50.16	3.97	12.99	2.33	16.16	101.1	19.0	74.2	61.1	24.4
2072-85	3021	51.21	4.35	12.75	2.42	14.66	107.3	18.0	74.8	61.1	26.1
2072-86	3252	53.93	4.03	13.05	2.40	16.21	104.6	17.9	74.3	61.1	25.4
2072-89	3164	51.28	4.11	12.92	2.61	15.13	101.8	18.3	73.0	60.6	24.9
2072-91	3021	50.91	4.07	12.85	2.68	15.09	107.3	18.1	75.2	59.3	25.2
2072-92	3072	52.46	4.11	12.93	2.57	15.38	107.0	17.6	74.2	61.1	26.4
Genotypes											
LSD											
$P = 0.05$	196	1.85	0.25	0.28	0.21	1.36	4.1	0.8	1.3	2.1	1.3
$P = 0.01$	264	2.48	0.34	0.38	0.29	1.82	5.5	1.1	1.8	2.9	1.8
CV%	5.27	5.06	7.40	2.99	12.75	12.24	4.01	4.01	1.75	3.77	6.09

Table 2 Regional trials conducted over three locations and 3 years: ANOVA *F*-test for genotype × environment interaction. NS = not signficant at $P = 0.05$; [NS] = significant at $0.10 > P > 0.05$; * = significant at $P = 0.05$; ** = significant at $P = 0.01$; *** = significant at $P = 0.001$. G = genotype; L = location; Y = year.

Interaction	Yield (kg/ha)	Grade index	Filling value (cc/g)	Moisture at equilibrium (%)	Total nicotine alkaloids (%)	Reducing sugars (%)	Plant height (cm)	No. leaves (per plant)	Days to flower	Leaf length (cm)	Leaf width (cm)
G × L	***	NS	NS	NS	NS	NS	**	NS	NS	NS	NS
G × Y	[NS]	NS	**	NS	NS	**	***	***	*	NS	NS
G × L × Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS

Table 3 Comparison of F_1 hybrid and mid parent value means conducted over three sites and 2 years: agronomic, chemical, physical, and field measurements.

	Yield (kg/ha)	Grade[a] index1	Grade[a] index2	Filling value (cc/g)	Moisture at equilibrium (%)	Total nicotine alkaloids (%)	Reducing sugars (%)	Plant height (cm)	No. leaves (per plant)	Days to flower	Leaf length (cm)	Leaf width (cm)
F_1 Hybrid	2897	12.25	14.02	4.71	12.86	2.28	20.34	117.1	17.3	70.1	66.1	27.8
Mid parent value	2784	12.39	13.94	4.89	12.75	2.18	20.26	115.9	17.4	71.4	64.4	27.6
Statistical contrast[b]												
F_1 vs parents	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS

[a] = Grade index of two tobacco manufacturing companies where grade index = weighted desirability based on a scale of 1–20 and related to grade-price schedule for each separate company.

[b] = *F*-test significance: NS = not significant at $P = 0.05$; * = significantly different at $P = 0.05$.

In flue-cured tobacco breeding programmes worldwide, use of the *N. glutinosa* TMV resistance has generally not met with success. The reason appears to be that during the transfer of the N gene from *N. glutinosa* (Holmes 1938) the intra-chromosomal segment also carries genes which result in TMV resistant *N. tabacum* breeding lines with inferior agronomic performance (Chaplin et al. 1961; Chaplin et al. 1966; Chaplin & Mann 1978). However, the results in the trials reported here indicate that 'Moskuaka 85', under New Zealand conditions, performs well in comparison with commercial cultivars lacking the N gene.

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Cytological examination of three cultivars of garlic (*Allium sativum* L.) by C-banding and flow cytometry

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Abstract C-banded karyotypes were prepared for three of the more important garlic (*Allium sativum* L.) cultivars grown in New Zealand, 'California Early', 'California Late', and 'Red Garlic'. The cultivars all have unique banding patterns with the largest bands at the sites of secondary constrictions. Further bands were located in terminal positions on different numbers of chromosomes in the three cultivars. A limited number of bands were seen in interstitial positions, especially on the nucleolar organiser (NOR) chromosomes. Centromeric bands were entirely absent. Flow cytometry was used to measure nuclear DNA amounts which were found to be the same in the three cultivars.

Keywords garlic; *Allium sativum* L.; Giemsa C-bands; 'California Early'; 'California Late'; 'Red Garlic'; flow cytometry

INTRODUCTION

Garlic (*Allium sativum* L.) is a diploid species that is always vegetatively propagated, as many cultivars never flower and those that do are completely sterile (McCollum 1976). Because they usually lack flowers, the number of characters available for the classification of cultivars is severely limited and use must be made of features such as leaf width and number, clove characteristics such as the colour of the paper tissue enclosing the cloves, and whether the cloves are formed in the axils of few (usually two) or many leaves. Other characteristics such as whether a flower stem forms or not, and water requirements are also considered important (Konvicka & Levan 1972), but are not always easily determined. A classification system for garlic has been devised by Hruby & Konvicka using these characteristics, and is cited in Konvicka & Levan (1972). The three New Zealand cultivars used in this study, 'California Early', 'California Late', and 'Red Garlic', can be placed, with some reservations, in each of the three proposed categories. An abbreviated version of their classification system is:

H Type. Cloves in the axils of two leaves, and the cloves have violet paper. Leaves are broad and are formed in a definite number. Flower stems usually develop. This category comprises late-maturing forms which have a high water requirement and remain upright when ripe. 'Red Garlic' falls into this category, but its paper colour is better described as red.

U Type. Cloves develop in many axils and the clove paper is white. Flower stems develop infrequently. Leaf number is indefinite. This category comprises later-maturing forms with broad leaves and is recumbent when ripe. It has a high water requirement. 'California Early' fits this category except that it is an early-maturing type.

A Type. This is similar to U Type in some respects, but it differs in early maturation, narrow leaves, and low water requirements. 'California Late' fits this category except that it is late maturing.

In this paper we have used Giemsa C-banding and the measurement of genome size by flow cytometry with the aim of finding other taxonomic characters to identify garlic cultivars. Giemsa banding can produce transverse bands on plant chromosomes by staining the regions of constitutive heterochromatin (Schweizer 1973; Vosa 1973). The mechanism of C-banding is still uncertain, but it is a

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useful technique that can be used to characterise crop cultivars or specific chromosomes in hybrids (Hesemann et al. 1987). Flow cytometry allows accurate measurements of a large number of fluorescent labelled nuclei by passing them through an excitation laser beam, causing individual nuclei to fluoresce. Using highly specific DNA stains, fluorescence emission intensity corresponds to nuclear DNA content. Measurements obtained correspond to relative DNA amounts which can be converted into absolute values by comparison with a standard of known DNA content. Thus different cultivars can be easily compared and any differences may be of diagnostic value. Recent studies on maize, for example, showed differences between cultivars (Rayburn et al. 1985) which can be related to their chromosome banding patterns and heterochromatin content.

MATERIALS AND METHODS

C-banding

Cloves of each of the cultivars were planted in a sterilised bark/soil mixture. Healthy root-tips were subsequently collected from vigorously growing plants. The excised root-tips were pretreated in a saturated solution of *p*-dichlorobenzene for 18 h at 4°C. They were fixed in a 3:1 solution of absolute ethanol:acetic acid for 24 h at 4°C. Chromosomes were prepared using the air-drying technique of Geber & Schweizer (1988). This involves the digestion of the cell wall with a mixture of pectinase and cellulase to produce protoplasts that can then be air-dried onto a slide. The chromosomes were then stained using the C-banding schedule of Seal & Bennett (1981). Slides were hydrolysed in 45% acetic acid for 2–4 min at 60°C, and then rinsed twice in distilled water. This was followed by treatment with a saturated solution of barium hydroxide (10 g in 200 ml at 60°C and then allowed to cool) at 20°C. After 5 min they were dipped in 1% acetic acid and rinsed in distilled water. The slides were then placed in 2×SSC (2× saline sodium citrate: 3.5 g sodium chloride + 1.76 g tri-sodium citrate in 200 ml distilled water) for 90 min at 60°C. The slides were rinsed again in distilled water, and then stained in a 3% (v/v) Giemsa's stain (Gurr's improved R66) in sodium phosphate buffer (pH 6.8) at room temperature for 10 min. The slides were then rinsed, oven-dried, and a coverslip mounted with DPX.

Flow cytometry

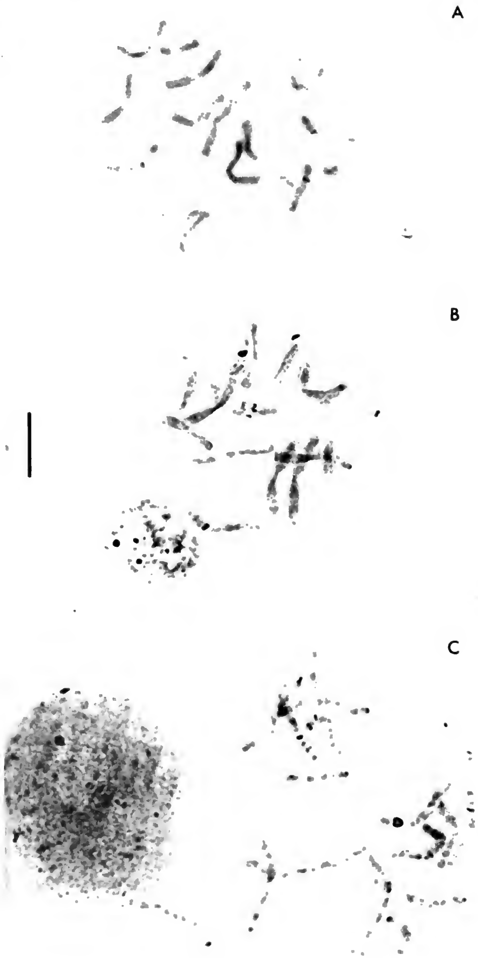
Healthy root-tips were collected from each of the three cultivars, and fixed for 20 min in ice-cold 4% formaldehyde in 'Tris' buffer (10 m*M* Tris (hydroxymethyl)-aminomethan, 10 m*M* disodium EDTA, 100 m*M* sodium chloride, pH=7.2) + 0.1% Triton X-100 (Levi et al. 1986). They were rinsed thoroughly in the buffer without fixative, and then finely chopped in the buffer with razor blades to release the nuclei. The suspensions were filtered through 64 µm and then a 16 µm nylon mesh filter. The nuclei were stained with 100 µg of propidium iodide ((3,8-diamino-5-[diethyl-methylammonio] propyl)-6-phenylphenanthridinium diiodide, Serva) per ml of sample. The samples were analysed using a Fluorescence Activated Cell Sorter (FACS) 440 (Becton Dickinson, Sunnyvale, U.S.A.) equipped with a Coherent Innova 90 argon ion laser. The collection photomuliplier was set to record emissions at a wavelength of 625 ± 17.5 nm, the emission peak of propidium iodide. Data were collected on a Microvax II computer. Graphs of the data were plotted by the Microvax II and average peaks of the graphs were estimated and measured.

RESULTS AND DISCUSSION

C-banding

The three cultivars had quite distinct banding patterns. 'Red Garlic' had the fewest bands with those on the nucleolar organiser (NOR) chromosomes being the most prominent (Fig. 1a,2a). Very small bands were present on three of the other chromosomes. 'California Late' (Fig. 1b,2b) showed more banded chromosomes than 'Red Garlic'. A slight asymmetry in the large bands was seen in the NOR chromosomes and this is reflected in the different sized chromocentres in the interphase nucleus (Fig. 1b). One pair of NOR containing chromosomes bore bands adjacent to the secondary constriction as well as other bands on the short arm. Of the remaining twelve chromosomes, only four appeared to have any bands and these were very small. The bands seen on 'California Early' (Fig. 1c,2c) were numerous and easily discernable compared with the other two cultivars. Of the two pairs of NOR chromosomes, one exhibited a very distinct asymmetry in band size. These bands were located at or near the secondary constriction and were the most distinctive feature of the karyotype. The other pair of NOR chromosomes also exhibited bands near to the

Fig. 1 C-banded mitotic chromosomes and interphase nuclei (B and C only) of *Allium sativum*. **A,** 'Red Garlic'; **B,** 'California Late'; **C,** 'California Early'. Scale = 10 µm.



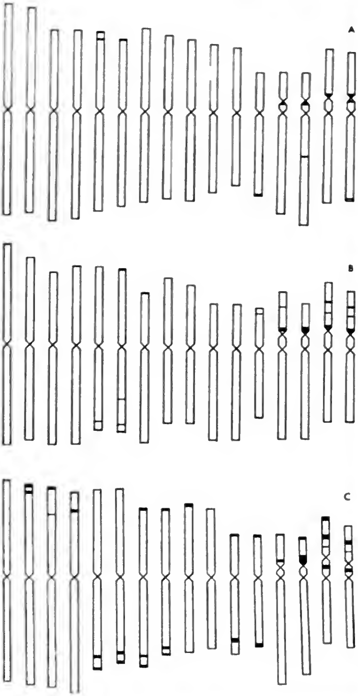


Fig. 2 Idiograms of the C-banded mitotic chromosomes of *Allium sativum*. **A**, 'Red Garlic'; **B**, 'California Late'; **C**, 'California Early'.

secondary constriction, as well as interstitial bands and a terminal band on the short arm of one of the chromosomes. The remaining 12 chromosomes in this complement exhibit terminal or near-terminal bands on one or both arms, although no bands were evident on two of the chromosomes. The more numerous bands in 'California Early' are reflected in the large number of chromocentres in the interphase nuclei (Fig. 1c).

Most published examples of C-banding in garlic do not specify the cultivar used, making comparisons difficult. Roy (1976) noted in populations of garlic from India, that the four chromosomes with secondary constrictions showed distinct bands, except in one population where only two of these four chromosomes revealed bands. The metacentric chromosomes showed bands in only one instance. Distinct heterozygosity was seen in the banding pattern both within and between populations examined. This suggested that the differences seen between the cultivars in his investigation may not be entirely cultivar-specific or that the cultivars are not truely clonal. In Spanish cultivars, Cortes et al. (1983) found bands in similar positions on the NOR chromosomes to those observed in this investigation, and in addition they found terminal bands on the short arm of one pair of metacentric chromosomes. An interesting feature noted by Cortes et al. (1983) and Cortes & Escalza (1986) is the presence of a secondary constriction and associated C-band on the long arm of the shortest pair of chromosomes. We have found no other reports of this additional pair of secondary constriction and have not observed them in New Zealand cultivars. Vosa (1977) reported only two pairs of chromosomes with secondary constrictions as was seen in this investigation, and this seems to be the most common arrangement in garlic. The sub-terminal band seen on the long arm of one of the short sub-metacentrics in 'California Early' is, however, in the same position as reported

Table 1 2C nuclear DNA values of *Allium cepa* 'Pukekohe Longkeeper' and three garlic cultivars measured by flow cytometry.

Sample	2C value per nucleus (pg DNA)	No. of cells measured
A. cepa 'Pukekohe Longkeeper'	33.50	10041
A. sativum 'California Early'	31.00	10902
A. sativum 'California Late'	31.00	5663
A. sativum 'Red Garlic'	33.00	5601

in the Spanish examples, and may be related in some way to the secondary constrictions and associated C-bands in the Spanish cultivars.

Flow cytometry

The results of nuclear DNA measurements by flow cytometry of the three garlic cultivars and an *Allium cepa* standard are given in Table 1. Our *A. cepa* standard is not the accepted standard cultivar (cv. Ailsa Craig is the normal standard (Bennett et al. 1982)) as this was not available for study. However, we thought it of value to include another *A. cepa* cultivar, 'Pukekohe Longkeeper', in our measurements and have assumed that it contains the standard DNA value of 33.5 pg/2C nucleus that is found in 'Ailsa Craig'. The values for 'California Early', 'California Late', and 'Red Garlic' are all very similar. 'California Early' and 'California Late' were both observed to have a peak between 30 and 31 pg DNA, whereas 'Red Garlic' peaked at a value between 32 and 33 pg DNA. This difference between cultivars is small, well within the range of machine error (± 5%) and consequently it appears that these cultivars all have the same nuclear DNA amounts. The published value for the 4C nuclear DNA amount of *A. sativum* is cited as 62.9 pg per cell by Bennett et al. (1982). This gives a 2C value of 31.45 pg of DNA per cell which corresponds closely with the values obtained here.

In conclusion, distinct differences in banding patterns were seen between these three cultivars, especially in the position of the bands relative to the secondary constriction on the NOR chromosomes, so for these three cultivars this would be a further useful means of identification. Despite the fact that there are differences in heterochromatin (C-bands) between cultivars we have found no differences in nuclear DNA content between cultivars.

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Comparison of four early-maturing Satsuma mandarins with the standard industry cultivar 'Silverhill'

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Abstract The Japanese Satsuma mandarin (*Citrus unshiu* Marc.) cultivars 'Okitsu', 'Miyagawa', 'Miho', and 'Matsuyama' were compar-ed with the standard industry cultivar 'Silverhill', over a 7-year period, at the Te Puke Research Orchard in the Bay of Plenty, New Zealand. Fruit on all four Japanese cultivars reached commercial maturity 3–4 weeks before 'Silverhill'. Their earliness, rounder fruit shape, and smoother rind, offer major advantages for potential export to Japan. Fruit quality (soluble solids (SS) content, SS:acid ratio (SS:A), juiciness) of 'Okitsu' was the highest of the Japanese group, followed closely by 'Miyagawa' and then 'Miho'. 'Matsuyama' had the poorest fruit quality (early loss of expressible juice, early onset of puffiness, bumpy fruit rind, and high variability). 'Okitsu' fruit had a mean annual SS concentration range and SS:A range of 8.1–9.0% and 5.7:1–7.0:1 respectively, over the 4-year period 1984–87. These fruit quality values were less than those for 'Silverhill' and may not be high enough for the Japanese market, or as high as is desirable for the domestic market. However, it should be possible to improve fruit quality by cultural techniques and/or by growing these cultivars in districts with a warmer climate.

Keywords Satsuma mandarin; *Citrus unshiu*; 'Okitsu'; 'Miyagawa'; 'Miho'; 'Matsuyama'; 'Silverhill'; fruit quality; soluble solids content; maturity; market

INTRODUCTION

The New Zealand citrus industry is undergoing significant reorganisation including a shift in emphasis towards export (Anon. 1985a). One focus of interest is in high-quality, easy-peel mandarins for which niches in overseas markets have been identified e.g., export of Satsuma mandarins (*Citrus unshiu* Marc.) to Japan in that country's off-season period.

Japan produces and consumes large quantities of Satsuma mandarins. In 1983, about 3 million t were produced whereas total citrus production amounted to 3.6 million t (Anon. 1985b). Outdoor harvest of early Satsuma mandarin cultivars begins in Japan in September and the season continues until March. The season can be brought forward to June by selecting early-maturing cultivars and growing these in plastic greenhouses (Glucina 1985). In Japan, there is a short gap between the end of the Satsuma season (March) and the beginning of the new season (June), providing a possible niche for New Zealand-produced Satsuma mandarins.

Satsuma mandarins are also popular on the New Zealand market because they are easy-peeling, seedless, and the first citrus of the season (Sale 1983). Until recently, the American selection 'Silverhill' (Hodgson 1967) was the only Satsuma mandarin cultivar available in New Zealand. However, its late maturity (i.e., May-July), relatively low soluble solids (SS) content, and oblate fruit shape, make it unsuitable for export to Japan. Interest has therefore focused on very early-maturing Japanese cultivars reputed to produce higher-quality fruit than 'Silverhill'.

This paper reports on the performance and characteristics of early-maturing Satsuma mandarin cultivars in New Zealand by comparing four Japanese

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selections with 'Silverhill'. These cultivars, selected in Japan for their early fruit maturity, have a similar origin: 'Miyagawa' originated as a limb sport of the cultivar 'Zairai'; 'Matsuyama' is a limb sport of 'Owari', and 'Okitsu' and 'Miho' are nucellar seedlings of 'Miyagawa'. 'Silverhill' is a nucellar seedling of 'Owari', selected in the United States (Hodgson 1967).

MATERIALS AND METHODS

The Satsuma mandarin cultivars 'Okitsu', 'Miyagawa', 'Miho', and 'Matsuyama' were introduced to New Zealand from the Okitsu Branch, Horticultural Research Station, Ministry of Agriculture, Forestry and Fisheries, Shizuoka, Japan, by W. A. Fletcher in 1970. Plants released from quarantine were used as a source of material to bud seedlings of trifoliata (*Poncirus trifoliata* (L.) Raf.) rootstock. 'Silverhill' plants of comparable size and age on trifoliata rootstock were obtained from a commercial citrus nursery.

Eight trees of each cultivar were planted in August 1977 at the DSIR Research Orchard in Te Puke, Bay of Plenty, New Zealand. The trial was laid out in two randomised blocks, each block consisting of five plots where each plot was a row of four trees. Plants were grown in well-sheltered conditions on a Paengaroa/Te Puke sandy loam soil and managed according to commercial practice. This included fruit-thinning (fruit to leaf ratio 1:25), adequate irrigation and nutrition, and disease and pest control.

Harvest period

Fruit were monitored each season from mid April to mid August but results are only presented for the period judged to represent commercial maturity. This was from the time of first complete colouring of the rind through to the onset of puffiness and dryness. Because the four Japanese Satsuma cultivars matured before 'Silverhill', results obtained with these cultivars are presented from 4 May to 10 July (six harvests in years 1984, 1986, and 1987; and five harvests in 1985), whereas 'Silverhill's' season was taken to be from 5 June to 25 July (five harvests in years 1984, 1986, and 1987; and four harvests in 1985).

Measurements

Fruit development was monitored from mid April to mid August over the 7 years, 1981–87 inclusive. Fruit SS values were determined with a hand-held Atago refractometer and measurements were corrected for acidity which was determined by titration with 0.1*N* sodium hydroxide (Dawes 1969). These measurements were taken on juice expressed from a 1-kg composite subsample of fruit, randomly selected from a 5-kg fruit sample harvested from a minimum of four trees of each cultivar on each harvest date. The SS:A ratio was calculated from these results. Expressible juice represented the volume of juice obtained by reaming 1 kg of halved fruit on a Sunbeam fruit juicer. Other fruit characteristics (shape, rind colour and thickness, peelability, and flavour) were recorded on similar representative samples, each season, by at least five people on each harvest date.

RESULTS

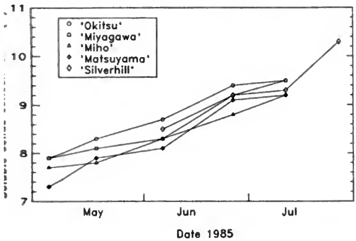
Only a small quantity of fruit was available during the initial trial period (1981–83 inclusive), and few significant differences occurred in these early years (data not shown). Tables 1-4 present mean annual data on SS, acidity, SS:A ratio, and expressible juice for the years 1984–87 inclusive. Typical comparisons of these properties for 1985, with values for each harvest date, are illustrated in Figs 1–4.

Soluble solids

The fruit SS content of the four Japanese cultivars had almost reached a maximum level at the end of June (Fig. 1) and increased only very slightly after this date. Although 'Silverhill' fruit coloured later (5 June) than the four Japanese cultivars, its SS content was similar to those of the four Japanese cultivars at this date. However, the SS content of 'Silverhill' did not reach a maximum level until late July. Although no significant differences in SS content of fruit were found among cultivars in 1984 and 1985 (Table 1), 'Silverhill' fruit tended to have the highest SS. In 1986 and 1987, significantly ($P<0.001$) higher SS were found in 'Silverhill' than in the other four cultivars. 'Miho' had the lowest SS in 1986 and 1987 and the values for the other cultivars were intermediate between 'Miho' and 'Silverhill'.

Acidity

Generally, fruit acidity levels (Fig. 2) decreased steadily over the harvest period. Except for 1987, there were significant differences in levels of acidity (Table 2) among cultivars. 'Silverhill' tended to have the lowest acidity level. Differences between 'Silverhill' and the other four cultivars were



ig. 1 Comparison of mean soluble solids concentrations corrected for acidity) in fruit of five Satsuma mandarin ultivars for 1985. (LSD = 0.82.)

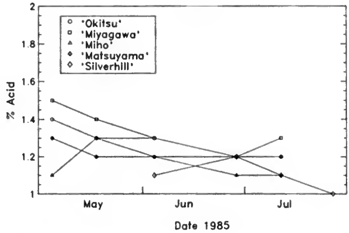


Fig. 2 Comparison of mean acid concentration in fruit of five Satsuma mandarin cultivars for 1985. (LSD = 0.1.)

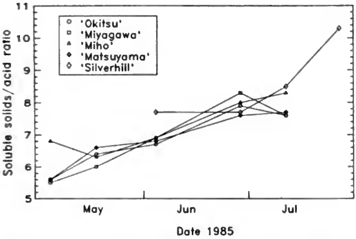


Fig. 3 Comparison of mean soluble solids/acid ratios in fruit of five Satsuma mandarin cultivars for 1985. (LSD = 1.14.)

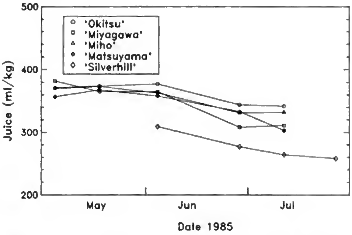


Fig. 4 Comparison of mean expressible juice values in fruit of five Satsuma mandarin cultivars for 1985. (LSD = 29.8.)

significant (1984: $P<0.05$; 1985: $P<0.001$; 1986: $P<0.05$).

SS:A ratio

SS:A ratios in fruit (Fig. 3) reached a maximum in all four Japanese cultivars by late June or early July. This date corresponded with the end of their harvest season. By contrast, 'Silverhill' fruit continued to show an increase in SS:A ratios until late July.

There were no significant differences in SS:A ratios of fruit (Table 3) among cultivars in 1986 and 1987, although 'Silverhill' tended to have the highest values. In 1984 and 1985, 'Silverhill' had a significantly ($P<0.05$) higher SS:A ratio than the other four cultivars tested, except 'Miho' in 1985.

Expressible juice

Juiciness in the fruit of four Japanese cultivars (Fig. 4) reached a peak as fruit began to colour in early May. This maximum was maintained during May and early June, but juiciness decreased thereafter. 'Silverhill' reached maximum juiciness in early June and decreased thereafter.

'Okitsu' had the most and 'Silverhill' the least (1984, $P<0.05$; 1985, $P<0.001$; 1986, $P<0.01$; 1987, $P<0.05$) expressible juice in all 4 years (Table 4). The other cultivars were intermediate between these two extremes. 'Matsuyama' tended to have the lowest juice content of the four Japanese satsumas.

Table 1 Mean annual soluble solids (%) concentration (corrected for acidity) in fruit of five Satsuma mandarin cultivars over the 4-year period 1984–87.

	Year			
Cultivar	1984 %SS	1985 %SS	1986 %SS	1987 %SS
Okitsu	9.0 a	8.9 a	8.1 bc	8.8 b
Miyagawa	9.1 a	8.6 a	8.7 b	8.7 b
Miho	8.9 a	8.3 a	7.6 c	8.3 b
Matsuyama	8.6 a	8.2 a	7.9 bc	8.8 b
Silverhill	9.6 a	9.3 a	9.8 a	9.7 a

Significance tests were based on separate SEDs calculated for each pair of means within a given year. This was necessary because of the unequal replication (times of testing). Values down a column with different letters are significantly ($P<0.001$) different.

Table 2 Mean annual acid (%) concentration in fruit of five Satsuma mandarin cultivars over the 4-year period 1984–87.

	Year			
Cultivar	1984 Acid %	1985 Acid %	1986 Acid %	1987 Acid %
Okitsu	1.6 a	1.3 a	1.3 ab	1.4 a
Miyagawa	1.7 a	1.3 a	1.4 a	1.4 a
Miho	1.6 a	1.2 bc	1.2 b	1.3 a
Matsuyama	1.6 a	1.2 ab	1.2 b	1.3 a
Silverhill	1.4 b*	1.1 c***	1.3 ab*	1.3 a

Significant tests were based on separate SEDs calculated for each pair of means within a given year. This was necessary because of the unequal replication (times of testing). Values down a column with different letters are significantly (* = $P<0.05$; *** = $P<0.001$) different.

Table 3 Mean annual soluble solids:acid ratio in fruit of five Satsuma mandarin cultivars over the 4-year period 1984–87.

	Year			
Cultivar	1984 SS:A	1985 SS:A	1986 SS:A	1987 SS:A
Okitsu	5.7:1 b	7.0:1 b	6.4:1 a	6.4:1 a
Miyagawa	5.5:1 b	6.7:1 b	6.2:1 a	6.7:1 a
Miho	5.6:1 b	7.3:1 ab	6.3:1 a	7.0:1 a
Matsuyama	5.9:1 b	6.7:1 b	6.7:1 a	6.8:1 a
Silverhill	7.2:1 a	8.4:1 a	7.4:1 a	7.6:1 a

Significance tests were based on separate SEDs calculated for each pair of means within a given year. This was necessary because of the unequal replication (times of testing). Values down a column with different letters are significantly ($P<0.05$) different.

Table 4 Mean annual expressible juice value (ml/kg) in fruit of five Satsuma mandarin cultivars over the 4-year period 1984–87.

	Year			
Cultivar	1984 ml/kg	1985 ml/kg	1986 ml/kg	1987 ml/kg
Okitsu	377 a	362 a	382 a	342 a
Miyagawa	342 ab	346 a	343 b	313 ab
Miho	368 a	354 a	355 ab	330 a
Matsuyama	343 ab	348 a	332 bc	296 b
Silverhill	317 b*	227 b***	308 c**	295 b*

Significance tests were based on separate SEDs calculated for each pair of means within a given year. This was necessary because of the unequal replication (times of testing). Values down a column with different letters are significantly (* = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$) different.

Table 5 Mean annual fruit weight (g) of fruit of five Satsuma mandarin cultivars over the 4-year period 1984–87. There were five replicates (times of testing) of each cultivar each year.

	Year			
Cultivar	1984 wt/fruit (g)	1985 wt/fruit (g)	1986 wt/fruit (g)	1987 wt/fruit (g)
Okitsu	79.3 a**	88.6 bc	107.4 a***	75.3 a***
Miyagawa	80.7 a	94.6 ab	95.2 b	77.9 a
Miho	66.9 c	83.3 c	91.6 b	68.8 b
Matsuyama	76.6 ab	101.8 a***	88.0 bc	81.0 a
Silverhill	70.1 bc	85.1 c	80.5 c	66.2 b

Values down a column with different letters are significantly (** = $P<0.01$; *** = $P<0.001$) different.

Other properties

Table 6 summarises additional observations made on important tree and fruit characteristics over the 7 years, 1981–87.

Tree characteristics

All cultivars produced commercial crops. 'Okitsu' was the most vigorous of the Japanese cultivars tested (Table 6), but overall the four Japanese cultivars were much slower-growing than 'Silverhill'. Significant (1984, $P<0.01$; 1985, 1986, and 1987, $P<0.001$) differences occurred between cultivars in fruit weight (Table 5), in all years 1984–87. However, the differences between the five

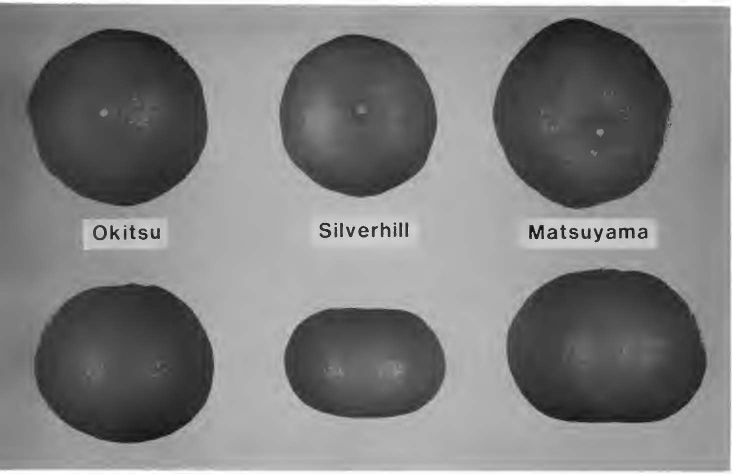


Fig. 5 Typical fruit of 'Okitsu', 'Silverhill', and 'Matsuyama', harvested at Te Puke in late May, indicating relative size, shape, and rind maturity differences.

cultivars were not consistent from year-to-year, except that 'Silverhill' tended to have a relatively low mean fruit weight.

Fruit appearance

Fruit of the Japanese Satsuma mandarins were rounder than 'Silverhill' and also had smoother rinds. 'Matsuyama' sometimes had a bumpy surface (Fig. 5). All cultivars had the same rind colour.

Peeling and pulp characteristics

Fruit of all five Satsuma mandarins were easy-peel types. Similarly, little difference in the adherence of albedo fragments to the surface of peeled fruit was noted. 'Silverhill' had an appreciably thicker rind than the four Japanese Satsumas (Table 6); therefore, fruit of the latter cultivars were observed to be more susceptible to withering than 'Silverhill'.

Fruit of all cultivars segmented well. 'Okitsu' had larger-sized segments whereas 'Silverhill' tended to have a larger number of smaller segments. Pulp colour and juiciness were similar with the exception that 'Matsuyama' appeared to lose juiciness earlier than the other cultivars. Fruit from the four Japanese Satsumas studied were informally judged to have low flavour, but 'Okitsu' was consistently preferred by tasters (Table 6). 'Silverhill' was judged to have the best flavour, and this observation was supported by data on SS:A ratios (Fig. 3). No differences in flesh texture were observed. Only an occasional seed occurred in fruit over the trial period.

DISCUSSION

Fruit of 'Okitsu', 'Miyagawa', 'Miho', and 'Matsuyama' reached full colour c. 3–4 weeks before 'Silverhill'. These dates at Te Puke were late April–early May for 'Okitsu' and 'Miho', early May for 'Miyagawa' and 'Matsuyama', and early June for 'Silverhill'. The four Japanese cultivars attained maximum fruit quality (SS, SS:A, juiciness) in mid June. However, fruit quality had deteriorated markedly by mid July when acidity and juiciness decreased and the rind became puffy. 'Silverhill', maintained good fruit quality up to the third week of July by attaining a high SS content and maintaining a high SS:A ratio. The Japanese cultivars tested, however, have substantial advantages over 'Silverhill' for potential export to Japan; they mature earlier, have a rounder shape, and have a smoother rind. These qualities are also proving to be desirable, for the beginning of the citrus season, on the domestic market.

Although overall fruit quality was not markedly different between 'Okitsu', 'Miyagawa', and 'Miho', 'Okitsu' fruit were slightly superior—they had more expressible juice, the best flavour, low segment number, the most vigorous tree habit, and were the earliest of the five Satsumas studied. 'Miho' was considered inferior to 'Okitsu' and 'Miyagawa' because of lower SS. 'Matsuyama' had the poorest fruit quality of the four Japanese cultivars studied because of early loss of expressible juice and onset of puffiness, bumpy fruit rind, and variable fruit quality.

The flavour of mandarins and oranges is indicated by the level of sweetness measured as SS and the SS:A ratio (Sinclair 1961). Maturity studies both in New Zealand and overseas indicate that when the SS:A ratio exceeds 7.5:1, fruit are acceptable to

Table 6 Summary of the main characteristics of five Satsuma mandarin cultivars over the 7-year period 1981–87.

	Cultivars				
	Okitsu	Miyagawa	Miho	Matsuyama	Silverhill
Yield[1]	9	9	9	9	9
Tree vigour[2]	6	4	5	3	10
Commercial harvest	late Apr –early May	early May	late April –early May	early May	early June
Fruit size	medium (60 × 50 mm)	small to medium (50 × 45 mm)	medium to large (65 × 55 mm)	medium to large (65 × 55 mm)	medium (60 × 45)
Fruit shape	near globose	near globose	near globose	near globose	oblate
Rind	smooth	smooth	smooth	sometimes bumpy	pitted
Rind colour[3]	orange (8–9)	orange (8–9)	orange (8–9)	orange (8–9)	orange (8)
Rind thickness	3–4 mm	3–4 mm	3–3.5 mm	3–4 mm	4–4.5 mm
Peeling characteristics	quite easy; albedo threads retained	fairly easy; some albedo retained	normally easy; albedo threads retained	easy; some albedo retained	easy; some albeo retained
Sectioning	fairly easy	easy	easy	fairly easy	easy
Segment no.	8–10	9–11	9–10	9–11	10–12
Pulp colour[3]	orange (9)	orange (9)	orange (9)	orange (8–9)	orange (9)
Juiciness	moderate	slightly low, but satisfactory	moderate	moderate initially, but dries quickly	moderate
Flavour	slightly tart, best Japanese cultivar	tart initially, low flavour	tart initially, low flavour	low flavour, variable, dries early in season	tart initially, reasonably good, best of 5
Pulp texture	chewy	chewy	chewy	chewy	chewy
Seed no.	nil	nil	nil	nil	nil

[1] Scale of yield, 1–10: 1 = very low; 5 = moderate; 10 = high.
[2] Scale of tree vigour, 1–10: 1 = very low vigour; 5 = low vigour; 10 = moderate vigour.
[3] According to standard Japanese colour charts (Yamazaki & Suzuki 1980).

consumers, but preferred flavours (described as "pleasant") are recorded when SS:A ratios range from 9:1 to 12:1. Similarly, oranges attain their best flavour in New Zealand when SS levels exceed 11%, but fruit are considered to be insipid below 9.0–9.5% SS (Fletcher & Hollies 1965).

In Japan, dessert citrus with SS:A ratios in the range 8:1–12:1 and SS >12%, are considered to be pleasant (Anon. 1985b). Such values are apparently achieved in Japan with late-maturing Satsuma mandarins, but early-maturing Satsumas on the Japanese market often reach only 9-10% SS (Glucina 1985). The fruit quality achieved with Satsuma mandarins in this study (i.e., mean annual SS:A ratio range of 7.2:1–8.4:1 and SS range of 9.3–9.8% for 'Silverhill'; 5.7:1–7.0:1 and 8.1–9.0% respectively for 'Okitsu', over the 4-year period 1984–87) is probably insufficient for the Japanese market and lower than desirable for the domestic market. This conclusion is confirmed by the low flavour rating of fruit of all cultivars tested. Only a relatively small increase in SS and SS:A values appears to be necessary, however, to match the quality of very early Satsuma mandarins currently available to Japanese consumers.

Mandarin quality, under New Zealand conditions, is greatly affected by total heat units available during the growing season (Sale 1988). In this respect, mandarins are very similar to oranges which have also been shown to improve in quality with an increase in heat units in New Zealand citrus districts. The heat indices available in northern New Zealand citrus-growing districts like Kerikeri (1795–2107) are much greater than at Te Puke (1347–1696) (Fletcher & Hollies 1965). Thus, a considerable improvement in the quality of Satsuma mandarins may be obtained in northern areas. Another option for improving fruit quality is to cover trees with plastic film. This cultural technique has produced promising results in preliminary studies at Te Puke (Martin 1989). Additional beneficial effects of the use of plastic covers appear to be earlier fruit maturity, increased tree vigour, and fruit size.

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Effects of forms and rates of organic and inorganic nitrogen fertilisers on the yield and some quality indices of tomatoes (*Lycopersicon esculentum* Miller)

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Abstract The effects of two organic (blood and bone, compost) and two inorganic (potassium nitrate, KNO_3; ammonium sulphate, $(NH_4)_2SO_4$) forms of nitrogen (N) fertilisers on the yield and some quality indices of tomatoes were studied in a pot experiment using Waimairi peaty loam soil obtained from an area currently used for vegetable production. Four rates (0, 150, 300, 600 kg N/ha, based on soil bulk density) of each fertiliser form were used. Fruit yield was significantly correlated with foliage N concentration, but not with fruit N concentration. Increasing rates of all N fertilisers, except compost, produced significant increases in fruit yield. Compared with the control, at 600 kg N/ha applied, compost increased fruit yield by 40% only whereas the other N fertilisers studied increased fruit yield by 120%. Fruit quality indices measured showed variable response to treatments. Fruit colour increased significantly on application of blood and bone, $(NH_4)_2SO_4$, and KNO_3, whereas compost showed no effect. Fruit vitamin C concentrations decreased when all N fertilisers were applied. Increasing N rate produced significant negative linear trends with vitamin C concentration decreasing by 18–28% depending on the form applied. Fruit size was unaffected by treatments but visual quality of fruit decreased when N in any form was applied.

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Keywords nitrogen fertilisers; organic; inorganic; tomato yield; quality indices; vitamin C; fruit size; colour

INTRODUCTION

Organic systems of producing vegetables and food have recently been attracting a great deal of interest from both the general public and potential producers. Many claims have been put forward as to the beneficial effects of organic nitrogen (N) fertilisers used in organic systems on crop quality (e.g., Vogtmann 1981). However, validations of these claims are based largely on theoretical extrapolation rather than scientific measurements.

Tomato yields have increased considerably in recent years (Hobson 1988). These increases may be attributed to genetic improvements and advances in cultural practices such as the use of N fertilisers (Adams 1986). Moderate applications of N fertilisers increase tomato yields (Adams et al. 1978) with optimum field rates usually between 75 and 100 kg N/ha. Few reports are available on the comparative yield responses of tomatoes to inorganic or organic N fertilisers, although Svec et al. (1976) observed no difference in tomato yields when plants were supplied with inorganic or organic N fertilisers.

While tomato yields have risen, emphasis on high productivity and uniformity seems to have been at the expense of many consumer quality attributes. A United States Department of Agriculture survey of consumer satisfaction reported tomatoes to have one of the highest dissatisfaction ratings (Stevens 1985). With respect to N fertiliser applications, many quality indices show no clear relationships. Variable effects have been reported for fruit size (Adams et al. 1976). Increasing rates of N fertiliser application have, however, been reported to decrease fruit vitamin C concentration (Penningsfield & Kurzmann 1961; Mengel 1979). Although organic N fertilisers are claimed to produce higher nutritional quality produce (Vogtmann 1981), other workers (Svec et al. 1976; Yoshida et al.

1984) have reported no significant effects of N fertiliser form on fruit vitamin C concentration.

The aim of the present study was to examine the effects of increasing rates of application of organic and inorganic N fertilisers on the fruit yield and some quality components of tomatoes.

MATERIALS AND METHODS

Pot trial

A 4 × 4 factorial design with four replicates, arranged in a completely randomised block was used. Pots were arranged in a glasshouse thermostatically controlled with a fan vent temperature of 25°C and a heat temperature of 15°C. Topsoil (0-150 mm) from a Waimairi peat loam (a Typic Medifibrists) was obtained from a commercial market garden. Some chemical properties of the soil and fertilisers used are shown in Table 1. These indicate an extremely high nutrient content in the soil (Bradenburg 1980). The soil was air-dried and sieved (<6 mm) before potting. Pots (200 mm deep × 190 mm diam., 4.5 litre capacity) were each filled with 4 kg of the soil and watered to a moisture content equivalent to 75% soil water-holding capacity (based on weight) with deionised water. After this, watering occurred when the soil moisture content declined to the equivalent of 60% water-holding capacity. An indeterminate tomato seedling (*Lycopersicon esculentum* Miller, cultivar 'Potentate') was transplanted into each pot before treatments were applied. Treatments used were four rates of N (0, 0.37, 0.75, and 1.55 g/pot, equivalent to 0, 150, 300, and 600 kg N/ha calculated on a bulk density basis for each of the two forms of inorganic-N fertilisers (potassium nitrate, KNO_3; ammonium sulphate, $(NH_4)_2SO_4$) and two forms of organic-N fertilisers (commercial blood and bone and a compost). All forms were applied to the surface of the soil. The compost consisted primarily of softwood sawdust mixed with chicke manure and other assorted organic matter. The material was left for 12–24 months being mechanically turned 2–3 times during this period This method was used to produce c. 200 t of compos (wet weight) annually for use on an intensiv vegetable property.

Harvesting, sampling, and analysis

Fruit was harvested as it ripened based on the disappearance of green (chlorophyll) pigmen (Grierson & Kader 1986). First harvest was obtaine 68 days after transplanting with the last harves occurring 13 days later. At harvest, individual frui were visually assessed (Table 2) and weighed, and fruit vitamin C concentrations were determine using the *N*-bromosuccinimide method (Barakat e al. 1955).

Fruit colour (lycopene concentration in ppm) was determined by blending c. 5 g of fresh fruit for 1 min in 20 ml acetone and 400 ml light petroleum and filtering through a small Büchner funnel. The extract was evaporated down and re-extracted with petroleum ether. Water present was removed using a separating funnel followed by the addition of $MgSO_4$ crystals. The remaining solution was made up to 25 ml and absorption determined at 470 nm on a Shimadzu spectrophotometer. Lycopene concentration was then calculated on the basis of the coefficient of extinction value (Barnes pers. comm.). Fruit N concentration was determined on a 0.25 g sample of freeze-dried and ground fruit using the modified Kjeldhal method (Bremner 1965). The same method was also used to determine foliage N concentration. Foliage was sampled opposite the first fruit truss, dried at 65°C, and ground to pass through a 1 mm sieve before N determination.

Table 1 Some properties of the soil and fertilisers used.

	C (%)	N (%)	C:N	P	K	Ca	Mg	S
				(g/100 g dry wt)				
Blood and bone	33	6.1	5:1	7.1	0.1	14.8	0.32	NA
Compost	37	2.0	19:1	2.7	1.6	7.6	0.51	NA
KNO_3	0	13.8	0.0	0.0	38.7	0.0	0.0	0.0
$(NH_4)_2SO_4$	0	21.2	0.0	0.0	0.0	0.0	0.0	24.3
Soil	13	0.9	15:1	270^{a}	25^{a}	27^{a}	52^{a}	35^{a}

NA = Not available.
^{a} = MAF Quick Test values.

Statistical analysis

A Genstat analysis of variance (ANOVA) program was used to determine the significance of treatments (form and rate) and their interactions. A Mini-tab package (Version 5.1) was used for detecting polynomial (linear and quadratic) trends. Standard errors of the means obtained were used to calculate protected Least Significant Differences (LSD) for forms at each rate applied. LSDs calculated from Mini-tab standard errors were adjusted for significant blocking effects as indicated by Genstat ANOVAs. Correction factors used were based on the equation below (Dyson pers. comm. 1988):

Correction factor =

$$\left(\frac{\text{Residual SS}}{\text{Residual SS} + (\text{Block SS} - \text{Residual MS} \times 3)}\right)^{-2}$$

RESULTS

Fruit yield (dry matter)

There was a significant ($P \leq 0.02$) interaction between form and rate of N fertiliser applied on fruit yield. Application of N as KNO_3, $(NH_4)_2SO_4$, or blood and bone resulted in the same positive linear increase in fruit yield, with maximum yields of c. 32 g/pot for all three forms when 600 kg N/ha was applied (Fig. 1). However, fruit yields obtained from compost were lower than those of all other fertiliser forms at the three rates of N applied, with compost application increasing yield significantly only at the highest N rate (600 kg N/ha.).

Mean fresh fruit weight

The form of N fertiliser or the rate at which it was applied showed no significant effect on the mean fresh fruit weight produced by each plant. Mean fruit weight ranged from 42 to 53 g per plant.

Visual fruit quality

The addition of N fertiliser decreased the visual quality of fruit significantly ($P \leq 0.004$) (Table 3). This effect was independent of form.

Vitamin C concentration

The rate of N fertiliser significantly affected ($P \leq 0.001$) the vitamin C concentration of fruit. The vitamin C concentration decreased linearly as application rates for all fertilisers increased (Fig. 2). The decrease was least when N was applied as KNO_3 or compost (18 and 19% reduction respectively) with blood and bone and $(NH_4)_2SO_4$ applications resulting in greater declines of 28 and 25% respectively.

Fruit N concentration

Fruit N concentration was significantly affected by form ($P \leq 0.001$) and rate ($P \leq 0.001$) of N fertiliser applied (Fig. 3). Whereas compost produced a small and variable effect on fruit N concentration,

Table 2 Rating scale used for assessing tomato fruit visual quality (Grierson & Kader 1986).

Scale	Description
9	Excellent, essentially no symptoms of deterioration
7	Good, minor symptoms of deterioration, not objectionable
5	Fair, deterioration evident, but not serious
3	Poor, serious deterioration, limit of saleability
1	Extremely poor, not usable

Table 3 Mean effects of rates and forms of N fertilisers on the visual quality of tomato fruit.

Rate (kg N/ha)	0	150	300	600	**
Visual quality	8.3	6.8	7.8	7.2	$LSD_{0.05} = 0.9$
Form applied	KNO_3	$(NH_4)_2SO_4$	Compost	Blood and bone	NS
Visual quality	7.1	7.5	7.8	7.8	

Rate × form = NS.
** = $P < 0.01$.

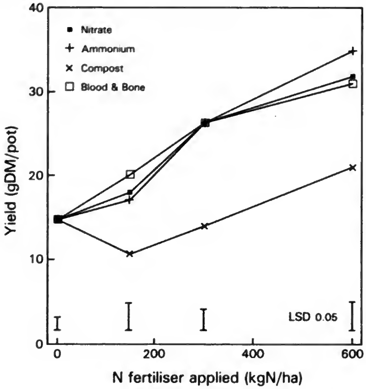

Fig. 1 Effects of rates and forms of two organic (blood and bone, compost) and two inorganic (potassium nitrate, ammonium sulphate abbreviated as nitrate and ammonium, respectively) N fertilisers on total tomato fruit yields (dry matter).

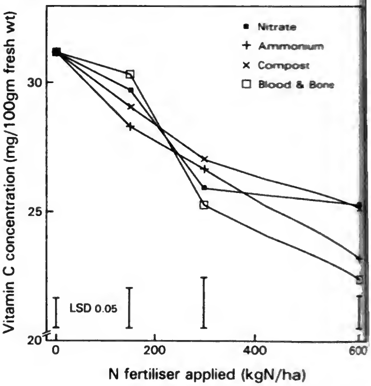

Fig. 2 Effects of rates and forms of two organic (blood and bone, compost) and two inorganic (potassium nitrate, ammonium sulphate) N fertilisers on vitamin C concentration of ripe tomato fruit.

both KNO_3 and blood and bone applications increased fruit N concentrations dramatically at 150 kg N/ha with smaller additional increases occurring at 300 and 600 kg N/ha. Fruit N concentration was also increased by $(NH_4)_2SO_4$ application but not to the same degree as KNO_3 and blood and bone.

Fruit colour (lycopene concentration)

The lycopene concentration of the fruit was significantly affected by form ($P \leq 0.035$) and rate ($P \leq 0.031$) of N fertiliser applied. Although compost application at any rate did not increase fruit colour, increasing rates of KNO_3, $(NH_4)_2SO_4$, or blood and bone resulted in significantly higher lycopene concentrations (Table 4).

Foliage N concentration

There was a significant ($P \leq 0.001$) interaction between form and rate of N fertiliser applied on foliage N concentration. Foliage N concentration was not affected by compost application. When N was applied as KNO_3, $(NH_4)_2SO_4$, or blood and bone, foliage N concentration increased linearly with increasing rates of fertiliser applied (Table 5).

DISCUSSION

The application of one organic (blood and bone) N fertiliser produced the same positive yield response from tomatoes as those produced by applications of both inorganic (KNO_3 and $(NH_4)_2SO_4$) N fertilisers (Fig. 1). This is related to the availability of N in the fertilisers applied (Goh & Vityakon 1983). The two inorganic N fertilisers studied released their N almost instantaneously on addition to the soil. The blood and bone appeared to behave similarly. Several workers (e.g., Haworth 1961; Svec et al. 1976; Nilsson 1979) have reported, for a range of vegetable crops, that organic N fertilisers produced yields similar to those produced by inorganic N fertilisers. However, N applied at all rates as compost produced yields significantly lower than those of other N fertilisers (Fig. 1). This poor yield response from compost application suggests that N in compost was not readily available to the plant (Goh & Vityakon 1983).

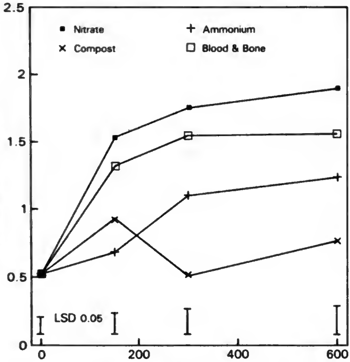

Fig. 3 Effects of rates and forms of two organic (blood and bone, compost) and two inorganic (potassium nitrate, ammonium sulphate) fertilisers on N concentration of omato fruit.

The difference between N supplied in compost and the other N fertilisers is also reflected in the lower foliage and fruit N concentration of plants fertilised with compost compared to those fertilised with the other N fertilisers (Table 5 and Fig. 3 respectively). Comparing the two organic N fertilisers, the significantly lower fruit yield obtained with compost as compared with blood and bone (Fig. 1), may be attributed to the lower N concentration and higher C:N ratio of compost (Table 1). Organic fertilisers with similar composition to the compost studied have been reported to show low and sometimes negative N availability over a 40-day period (Rubins & Bear 1943). These workers also reported that organic compounds with similar N concentration and C:N ratio to that of the blood and bone used in the present study, showed 50-60% of the organic N to be mineralised after 40 days. Although the present study was not conducted under the same conditions as those of Rubins & Bear (1943), it is likely that an even greater percentage of blood and bone N may have been mineralised in the present study because of the longer period (70-80 days) and favourable environmental conditions existing in the glasshouse trial. This may have resulted in more N from blood and bone being available to the plant and hence the observed higher yields from blood and bone compared to compost at equivalent N application rates.

The physical state of the two organic N fertilisers studied may also be a contributing factor to the differing yield responses. Blood and bone was finely ground whereas the compost contained predominantly coarse materials. Thus, blood and bone with a higher surface area would be expected to be more readily attacked by soil micro-organisms leading to rapid mineralisation of organic N.

The significant positive linear relationships obtained between yield and rate of N fertilisers applied other than compost suggest that a maximum yield had not been reached under glasshouse conditions even though a field equivalent rate of

Table 4 Effects of rates and forms of N fertilisers on tomato fruit colour (g lycopene/g fresh wt).

Rate (kg N/ha)	0	150	300	600	Mean of forms
Form applied					
KNO_3	16.8	25.7	20.9	26.6	22.5
$(NH_4)_2SO_4$	16.8	19.5	22.3	19.3	19.5
Compost	16.8	17.8	15.1	19.7	17.3
Blood and bone	16.8	17.8	25.1	22.3	20.9
Mean of rates	16.8	20.1	20.9	22.0	
Rate	**		$LSD_{0.05}$ = 3.5		
Form	**		$LSD_{0.05}$ = 3.5		
Rate × form	NS				

** = $P < 0.01$; NS = not significant.

Table 5 Effects of rates and forms of N fertilisers on N concentration (%) of tomato foliage.

Rate (kg N/ha)	0	150	300	600	Mean of forms
Form applied					
KNO_3	1.2	1.3	1.6	2.1	1.5
$(NH_4)_2SO_4$	1.2	1.3	1.5	2.0	1.5
Compost	1.2	1.2	1.2	1.3	1.2
Blood and bone	1.2	1.3	1.4	2.1	1.5
Mean of rates	1.2	1.3	1.4	1.9	
Rate	***		$LSD_{0.01}$ = 0.2		
Form	***		$LSD_{0.01}$ = 0.2		
Rate × form	***				

*** = $P < 0.001$.

600 kg N/ha was applied. This highlights the difference between the present pot trial and field conditions, where optimum rates of 75–100 kg N/ha have been reported for tomatoes (Adams et al. 1978). These differences may result from the favourable glasshouse environmental conditions and the difficulty of calculating field equivalent rates when applied to pot conditions. In any event, pot trial results should not be directly transferred to the field.

The form of N fertiliser applied did not affect measured quality indices other than fruit quality. Fruit colour (lycopene concentration) appeared to be directly influenced by N fertilisers. Whereas compost application did not affect fruit colour, application of other N fertilisers resulted in an increase in lycopene concentration (Table 4). This disagrees with results reported by Ellis & Hamner (1943) who found lycopene concentrations were unaffected by fertiliser applications. The application of N fertilisers has, however, been reported to increase the concentration of chloroplasts, the sites of lycopene synthesis during fruit ripening (Mengel 1979). Although chloroplast concentrations were not directly measured, fruit N concentration (an indicator of chloroplasts) showed a similar response to N fertiliser applications as did lycopene concentrations in fruit (Fig. 3). This may account for the observed increase in lycopene concentration in response to N fertiliser additions.

The rate of N fertiliser applied significantly decreased vitamin C concentration (Fig. 2) and fruit visual quality (Table 3). The lack of any difference between the form of N fertiliser applied suggests these indices are not directly related to N. Compost with its apparent lower N availability produced the same decrease in measured fruit quality indices as blood and bone and the two inorganic N fertilisers studied.

CONCLUSION

Although the application of one organic N fertiliser (blood and bone) produced the same positive tomato yield responses as applications of equivalent rates of two inorganic N fertilisers (KNO_3, $(NH_4)_2SO_4$), compost produced significantly lower yields at all rates of N applied. Most indices of fruit quality did not alter as the form of N applied changed. However, tomato fruit quality indices measured were reduced with increasing rates of all forms of N fertilisers applied, the exception being lycopene. These results show that claims of higher quality produce arising from the use of organic compared with inorganic N fertilisers were not validated in the present study.

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Composition of New Zealand apricot kernels

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Abstract The proximate analysis of 'Moorpark' apricot kernels (*Prunus armeniaca*) has been determined. Moisture (4.7%), protein (20.6%), dietary fibre (2.5%), total ash (2.9%), and lipid (52.0%) values were obtained. A potentially valuable oil was extracted and chemically analysed. The fatty acid composition, measured by gas chromatography of the methyl esters, was dominated by oleic acid (69.0%) and linoleic acid (26.0%). Cyanide was not detected in the apricot oil.

Keywords apricot kernels; apricot (*Prunus armeniaca*); apricot kernel oil; nitrogen content; lipid content; fatty acid composition; dietary fibre; ash content; cyanide content

INTRODUCTION

Apricots (*Prunus armeniaca*) are an important fruit crop in the Central Otago region of New Zealand. Disposal of apricot pits (kernel and endocarp) currently represents a minor problem for Central Otago apricot processors. However, recent reports (Foster & Elvidge 1984; Jarvis 1985; Anon. 1988) have indicated that kernels for disposal in the region will increase by c. 68% to a total of 40 t by 1990. Previous workers have indicated that there is a significant proportion of oil (c. 50%) and other compounds such as benzaldehyde, surface active agents, and proteins in Egyptian apricot kernels (Abd El-Aal et al. 1986a, b). Indian cultivars are reported (Joshi et al. 1986) to contain c. 44% oil.

This investigation has been conducted on kernels from 'Moorpark' apricots to confirm and compare the composition with previously reported data on other varieties. It may also assist in the assessment of the commercial viability of oil extraction from apricot kernels. Extraction of useful substances may provide a means of extending the relatively short processing seasons in fruit growing areas which may assist in reducing the fixed cost elements of equipment and facilities for processors.

MATERIALS AND METHODS

Apricot kernels

Apricot pits were obtained from a Central Otago fruit processing organisation. They were collected during January and February of the same year and were from 'Moorpark' apricots (*Prunus armeniaca*) harvested in January 1987. They were transported in polypropylene mesh sacks and held at c. 3°C until required. Kernels were removed from the endocarp by hand. As required, kernels were dried to constant weight in a convection oven not exceeding 40°C.

Samples for analysis (600–700 g)were selected at random from all sacks. These were then subsampled at random (100 g) and blended in a coffee grinder immediately before analysis.

Extraction of oil

Bulk lipid samples were extracted using a standard Soxhlet extraction system with chloroform methanol (2:1 v/v) as solvent. Once the solvent had been removed by rotary evaporation the lipid fraction was washed three times with distilled water to remove surface active material.

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Analytical methods

Nitrogen was determined in quadruplicate on 0.1 g samples using the micro-Kjeldahl method and protein was estimated using a factor of 6.25.

Dietary fibre was determined in quadruplicate using the neutral detergent method described by Van Soest & Wine (1967) on 1 g samples. The total carbohydrate (excluding dietary fibre) was calculated by difference.

Ash was estimated four times on each sample (0.5 g). After pre-ashing, samples were heated to 550°C for 24 h.

Lipid content was estimated four times on 0.2 g samples using the Soxtech semi-automatic extraction apparatus using chloroform methanol (1:2 v/v) solvent. After solvent was removed by evaporation, the oil was filtered and washed three times with excess water. AOAC (1984) methods were used for Iodine number and Saponification values. The defatted flour was treated at 40°C for 16 h to remove solvent and retained for further analysis.

Methyl esters of fatty acids were prepared by treating 8 mg of oil in 0.4 ml toluene with 0.5 ml boron tetrafluoride-methanol in methanol (1:3 w/v) in a boiling bath for 30 min in sealed vials. Methyl esters were extracted using pentane (4 ml) and redissolved in diethyl ether for GC separation of components. A Shimadzu CG 5A was fitted with 1.75 × 0.003 m glass column containing 3% ECNSS-X on Gas Chrom Q and it was run isothermally at 90°C. Nitrogen was the carrier gas at 35 ml/min and the equipment was fitted with a flame ionisation detector. Peaks were identified by co-chromatography using prepared standards or prepared standard mixtures (Sigma).

The AOAC (1984) qualitative determination for cyanide was done in duplicate on 2 g samples of defatted flour and on 2 g samples of apricot oil.

RESULTS AND DISCUSSION

Table 1 lists the physical and chemical characteristics of apricot kernels. The mass of kernels found in 'Moorpark' apricots is higher than that previous reported (Abd El-Aal et al. 1986a) for the Egyptian 'Amar' variety although it is a smaller proportion of the whole pit (25%).

Protein concentration of 'Moorpark' apricot kernels is lower than that of 'Amar' (24.1%), but comparable to that of other unnamed Egyptian varieties (22.2 and 23.1%) when allowance is made for moisture (Salem & Salem 1973). The protein content for 'Moorpark' kernels is comparable to those found (Joshi et al. 1986) for two Indian cultivars and compares favourably (Abd El-Aal et al. 1986a) with the protein content of such food groups as cereals. Furthermore, in vitro studies have demonstrated that there is comparable digestibility to standard protein casein in pepsin, trypsin pancreatin, and pancreatin-pepsin systems (Abd El-Aal et al. 1986a) and it may also have useful foaming and emulsification properties.

Table 1 Comparison of composition of 'Moorpark' apricot kernels with literature values.

	Moorpark				Egyptian	Egyptian		
Property	Mean	Range	SD	Amar[a]	Sweet[b]	Bitter[b]	Morpankhar[c]	Chavaru[c]
Mass of 100 pits (g)	255.6			137.2	ND	ND	ND	ND
Mass of 100 kernels (g)	65.1			42.8	ND	ND	ND	ND
Moisture content (%)	4.7	0.15	0.06	2.8	0	0	5.5	3.4
Nitrogen content (%)	3.29	0.12	0.05	3.86				
Protein content (N × 6.25) (%)	20.6	0.80	0.05	24.1	23.10	22.20	21.5	20.2
Crude fibre (%)	2.5	1.08	0.45	2.4	2.74	2.29	16.4	18.0[d]
Lipid content (%)	52.0	2.8	0.11	50.9	53.17	55.96	44.2	44.6
Ash (%)	2.9	0.24	0.11	2.2	2.47	2.15	2.8	2.3
Cyanide	present			0.15		present		
Total carbohydrate (%) (excluding dietary fibre)	17.3			17.5	18.52	17.40	9.4	10.8

[a] Values obtained by Abd El-Aal et al. (1986a, b).

[b] Values obtained by Salem & Salem (1973) and are quoted on a dry weight basis.

[c] Values obtained by Joshi et al. (1986).

[d] Crude fibre.

SD = Standard deviation.

ND = Not determined.

The dietary fibre content of the 'Moorpark' kernels was 2.5%, which is comparable to that found for the three Egyptian kernels but much less than he crude fibre values of 16.4 and 18% obtained for Indian cultivars. It is possible that the defatted flour remaining after industrial oil extraction may provide a digestible animal feed provided that hydrocyanic acid content is not high. In our study cyanide was detected but not quantified in the defatted flour. Previous workers (Stoewsand et al. 1975) have reported that the cyanide content of dried apricot kernels range from 11.7(0.5) mg/100 g for Moorpark' apricots to 177.1(11.1) mg/100 g for Summerland' apricots.

The lipid content of the 'Moorpark' kernels of 52.0% was similar to that found for Egyptian 'Amar' kernels (Abd El-Aal et al. 1986a) (allowing for moisture) but less than that found in other Egyptian cultivars (Salem & Salem 1973). The lipid content of the Indian kernels is noticeably lower. Repeated washing of the 'Moorpark' oil with water (×3) left a residue of 50.8% neutral lipid. Cyanide was not detected in the crude lipid extract.

The ash content of 'Moorpark' kernels is comparable to that found by other workers (Table 1). The total carbohydrate content excluding crude fibre was comparable to that found for the three Egyptian kernels but considerably more than that reported for Indian cultivars.

The fatty acid composition of the neutral lipid fraction from the 'Moorpark' apricot kernels is given in Table 2. For comparison, the fatty acid composition of oils from kernels of apricots grown in other countries, is included in Table 2. The 'Moorpark' kernel oil resembles that of the Egyptian 'Amar' cultivar (Abd El-Aal et al. 1986b). Except for the lower myristic and stearic acid values the 'Moorpark' oil is comparable to that found from sweet Morpankha and bitter 'Chavaru' Indian apricot kernels (Joshi et al. 1986). The 'Moorpark' results are similar to those found for the French cultivar (Farines et al. 1986) and shows some similarities with the Israeli cultivar (Gutfinger et al. 1972) and the Japanese ume apricot kernel (*Prunus mume*) (Ogihara et al. 1982). It would be surprising if there is exact agreement with literature values for a number of reasons, including the observation of Ogihara et al. (1982) who found the composition of the kernel changed during fruit development and maturation. Furthermore, the influences of soil type, geographical location, and seasonal variation were not assessed. The results may be of special interest to oil processors since the value of imported vegetable oils outstripped export values for exported oil by $27.9 million during 1987 (Anon. 1988). The total quantity of edible oils imported into New Zealand was c. 3 million litres. The apricot oil which is expected to be available from Central Otago may offset the cost of imports. A previous report (Abd El-Aal et al. 1986b) has in-dicated that performance characteristics of 'Amar' apricot oil allow substitution for traditional edible oils, such as peanut and soybean oils. Properties of 'Moorpark' apricot oil will be the subject of continuing study at the University of Otago. Furthermore,

Table 2 Fatty acid composition of the neutral lipid fraction of apricot oil.

Fatty acid	Moorpark						Rouge du		Ume[e]
(% of total fat)	Mean	Range	SD	Amar[a]	Morpankha[b]	Chavaru[b]	Roussillon[c]	Raananad	(*Prunus mume*)
Myristic	Tr			Tr	1.29	1.10	Tr		Tr
Palmitic	4.4	2.2	0.19	4.37	4.11	3.50	4.8	6.2	8.8
Palmitoleic	0.3	0.02	0.01	0.12	Tr	Tr	0.7	0.6	1.2
Stearic	0.2	0.01	0.01	0.46	2.07	1.98	0.8	0.8	1.4
Oleic	69.0	3.4	1.4	66.29	69.30	73.38	62.1	72.9	56.5
Linoleic	26.0	1.3	0.7	28.64	23.23	20.04	31.6	19.5	31.7
Linolenic	0.1	0.01	–	0.12	–	–			0.2
Iodine number	105.3			103.8	104.10	102.01	108		
Saponification number	187.7	189.7							

Tr = Trace.

[a] Values obtained by Abd El-Aal et al. (1986b).

[b] Values obtained by Joshi et al. (1986).

[c] Values obtained by Farines et al. (1986).

[d] Values obtained by Gutfinger et al. (1972).

[e] Values obtained by Ogihara et al. (1982).

SD = Standard deviation.

the remaining defatted flour may be of use as an animal feed.Future work will also consider the concentration of such compounds as complex polysaccharides which may be used as food conditioners.

It is possible that the short season enjoyed by apricot processors may be extended by short-term storage of pits for subsequent extraction. Such activity may provide a means of increasing equipment usage. If successful this will assist in reducing fixed costs associated with stonefruit processing.

CONCLUSIONS

The analysis of 'Moorpark' apricot kernels gave results that were comparable with those obtained for apricot kernels produced in other countries. 'Moorpark' apricot kernels are composed of c. 50% oil which is readily extracted. From its fatty acid composition the oil appears to be suitable as a partial replacement for vegetable oils. Cyanide was not detected in the lipid extract.

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rapid, simple test for heat damage in durum wheat

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bstract A simple, rapid, diagnostic test developed or the detection of heat damage in bread wheat was hown to be suitable for a variety of durum wheat ultivars from New Zealand and Australia. This rotein solubility test (PST) quantitatively measures he loss of solubility of albumins and globulins in eat-damaged wheat. The PST clearly distinguished ound from heat-damaged durum wheat that would ause problems in baking, seed germination, and lough-mixing and extrusion for pasta manufacture. n 1988, a New Zealand milling company used the ST to check lines of artificially dried durum wheat t the time of wheat delivery. No heat-damaged amples were found by the test or subsequently as a roblem during the manufacture of pasta.

eywords protein solubility test; durum wheat; read wheat; heat damage; baking; germination; asta manufacture; dough mixing; extrusion; wheat rying; mill wheat intake

INTRODUCTION

There is very little published information on the onsequences of overheating durum wheat during rtificial drying or storage of moist grain. Also, no simple, rapid tests for heat damage to durum wheat have been reported. Coleman & Rothgeb (1927) demonstrated that heating moist durum wheat at 38°C for 90 days to simulate the conditions of "bin-burned" wheat, produced the typical properties of heat-damaged wheat, i.e., reduced gluten extractability, small loaf volume, and poor crumb texture. Drying moist durum wheat artificially at 82°C in a cross-flow grain dryer caused a problem of grain breakage (Backer & Larson 1986). It is generally accepted that pasta cooking quality is associated with quality and quantity of durum semolina proteins (Grzybowski & Donnelly 1979; Autran & Berrier 1984). Also, gluten strength in durum wheat influences dough-mixing properties of semolina and cooking quality of spaghetti (Dexter et al. 1981). The loss of gluten strength resulting from the denaturation of gluten proteins by overheating moist durum grain during artificial drying might therefore produce serious problems for pasta manufacture.

In New Zealand, the moisture content of durum wheat near harvest is sometimes at a level that results in a risk of pre-harvest sprouting. To reduce the risk of sprouting in these seasons, the moist durum wheat is harvested and dried artificially rather than dried in the field. Thus, there is a need for a rapid, simple, inexpensive test to detect thermal denaturation of proteins in artificially dried grain that might lead to loss of quality of durum wheat. Recently, a simple, rapid protein solubility test (PST) was developed for the detection of heat damage to bread wheats (Every 1987). This paper investigates the use of this test for durum wheats and estimates its value in predicting loss of quality of heat-damaged durum wheat.

METHODS AND MATERIALS

Grain samples

New Zealand durum wheat cultivars, grown on farms in mid and south Canterbury, included: one 'Vernum' sample from the 1984 harvest year; one 'Tara' sample from the 1985 harvest year; and nine

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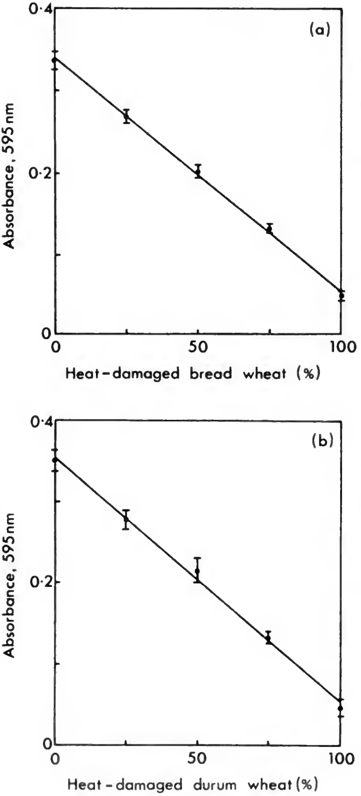

Fig. 1 Relationship of heat damage in wheat to protein solubility as measured by the PST. Wholemeals from totally heat-damaged wheat were blended with sound wholemeal from the same source and analysed in duplicate. (a) PST standard curve for bread wheat. The points in the figure show mean values of six determinations on each standard wheat blend made at different times over a 2-week period. The bars show standard deviations. (b) PST standard curve for durum wheat. The points in the figure show mean values for the 16 durum wheats listed in the Methods section. The bars show standard deviations.

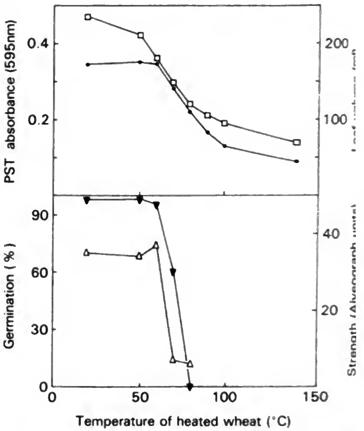

Fig. 2 Comparison of PST (●, absorbance), baking test (☐ loaf volume), germination test (▲; %) and alveograph test (△, strength; area under alveogram curve × 1.4) for detection of heat damage in durum wheat heated at various temperatures. The points in the figure show mean values of duplicate determinations.

'Tara' and three 'Aldura' samples from the 1987 harvest year. Australian durum wheat cultivars grown in Australia included 'Kamilaroi' and 'Yallaroi' from the 1988 harvest year. All the samples were naturally dried in the field to moisture contents below 14%.

The New Zealand bread wheat cultivar, Oroua, from the 1986 harvest season, was used as the standard wheat in the PST for heat damage (Every 1987). This sample had an absorbance value (0.350) for the PST which represented an average value determined for 34 bread wheats representing 27 cultivars from six countries throughout the world.

Protein solubility test for heat damage in wheat

The reduced solubility of heat-damaged wheat proteins in aqueous NaCl was utilised to develop the PST (Every 1987) which is summarised as follows. Wholemeal flour (1 g) in a 100 ml measuring cylinder was mixed with 2% (w/v) NaCl (50 ml) by inversion once a sec for 30 sec. The flour was left to settle for

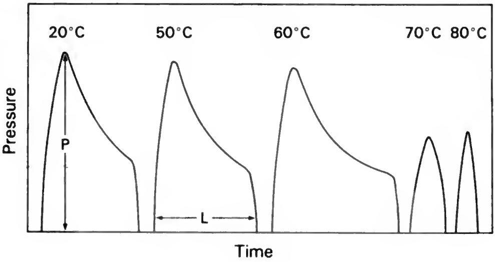

Fig. 3 Typical alveograms for durum wheat samples heated at 20, 50, 60, 70, and 80°C from the left. Maximum overpressure (P) and average abscissa at rupture (L) are shown.

10 sec, then 0.1 ml of extract was removed from the top layer of liquid and mixed with 3 ml of Bradford (1976) protein stain reagent. The absorbance at 595 nm was measured after 2 min against a blank of protein stain reagent. At the Wheat Research Institute, a Falling Number Mill Type 3303 set at zero was used to prepare wholemeal flour for the PST standard curve (Fig. 1). At the commercial mill laboratory, a Falling Number Mill Type 3100 was used to prepare wholemeal for the PST.

Heating procedures

Large samples (500g) of durum wheat (cv. Tara, 1987) suitable for test-baking and alveograph tests were conditioned to 18% moisture content for 16 h before heating. Samples were spread out in a thin layer in plastic oven bags, sealed, and heated in laboratory ovens for 1 h at 50, 60, 70, 80, 90, 100, or 140°C. Samples were air-dried to less than 14% moisture content before testing.

Multiple samples of totally heat-damaged wheat for the PST standard curves were prepared on a small scale as described by Every (1987). Wheats (40 g) of natural moisture content were mixed with distilled water (0.8 ml) in pyrex tubes (32 mm i.d.), capped with aluminium foil, and heated for 1 h at 150°C in an aluminium heating block. The loaf volume of bread baked with totally heat-damaged wheat has a limiting value of 25–30% of control (Every 1987).

Germination test

After soaking wheat for 2 h in distilled water, 100 kernels were placed on moist filter paper in a Petri dish and kept at 20°C away from light. The proportion (%) of wheat germinated was recorded at 7 days.

Baking test

Bread was made by the Wheat Research Institute 50 g mechanical dough development process (Swallow & Baruch 1986). White flour was prepared on a Buhler MLU-202 laboratory mill. Doughs were produced from 50 g of white flour using optimum water absorption and work input values as determined on a 125 g Mitchell Mixer (Mitchell 1984) for the sound durum wheat being tested. The dough was proved for 8 min at 32°C, moulded, proved again for 45 min at 40°C, then baked for 25 min at 210°C. Loaves were left overnight at room temperature (20°C) before assessment of volume by rape seed displacement.

Chopin Alveograph

Tests were performed in duplicate on the older model Chopin Alveograph as described in the Alveograph handbook (Faridi & Rasper 1987). White flour was prepared on a Buhler MLU-202 laboratory mill.

RESULTS AND DISCUSSION

Relationship of protein solubility to heat damage

The PST standard curves for bread wheat and durum wheat are presented in Fig. 1. A comparison of these standard curves by an analysis of covariance showed they were not significantly different ($P = 0.01$). The data were pooled to give the functional linear equation (Berkson's case; Berkson 1950);

$$A = 0.349 - 0.00295\,H$$

where A is the absorbance from the PST, and H is the percentage of heat-damaged wheat for both bread and durum wheat. The coefficient of determination (r^2) was 99.7%. Thus the same PST standard curve can be used for the quantitative determination of heat damage for both bread and durum wheats.

Comparison of protein solubility test, baking, germination, and alveograph tests

The PST was compared to three other tests that can determine changes in wheat quality caused by heat damage (Fig. 2). The baking test (Coleman & Rothgeb 1927), germination test (Hutchinson et al. 1946), and alveograph test (Tosi et al. 1986) have all been used to determine heat damage in bread wheat.

Cultivar Tara was chosen for the tests because other durum wheat cultivars are rarely grown in New Zealand.

All the tests showed drastic changes in durum wheat quality in samples heated above 60°C. Although there was some loss in baking quality (loaf volume) of durum wheat heated at 50 and 60°C which was not reflected by the PST, the two tests generally showed comparable changes for the different heating temperatures. Durum wheats are, however, not generally used for baking bread and it may be more appropriate to compare the PST to alveograph tests. The Chopin Alveograph is often used to provide information on the characteristics of durum wheat for its most common usage in the manufacture of pasta (Cubadda 1988).

The alveograph strength measurement (area under alveogram curve × 1.4) dropped drastically in wheats heated above 60°C and this coincided with the drop in PST values (Fig. 2). This corresponded with a drastic drop in both the dough tenacity values (maximum overpressure, P) and dough extensibility values (average abscissa to rupture length, L) for samples heated above 60°C (Fig. 3). Samples heated at 70°C were extruded very slowly by the Alveograph and consequently became overmixed. Samples heated at 80°C were extremely difficult to extrude and samples heated at higher temperatures could not be extruded at all. These results showed that heat-damaged durum wheats would cause mixing and extrusion problems in the manufacture of pasta products and the PST was able to detect heat damage at a level that would cause these problems. The weak gluten properties of heat-damaged durum wheat might also produce pasta of poor cooking quality because it is considered that durum wheats with strong gluten properties tend to produce pasta with superior cooking characteristics (Dexter et al. 1981; Cubadda 1988).

Practical use of the protein solubility test

Wet weather was experienced at critical times during the 1988 harvest period for durum wheat in New Zealand. To avoid the possibility of pre-harvest sprouting, many lines of durum wheat were harvested before fully dried in the field (mostly about 18% moisture content) and then artificially dried to below 14% moisture content. All the artificially dried wheat lines (272) were analysed by the PST at the time of wheat delivery at the mill to ensure that no heat-damaged lines were stored in the mill silos. Some of the PST methods used by the mill laboratory were different from those used by the Wheat Research Institute for Fig. 1. Different Falling Number Mill Types, mixing methods, and spectrophotometers were used. Although these changes gave higher absorbance values (in the order of one absorbance unit) than those shown in Fig. 1, the measurements were consistent. This verified the results of Every (1987)which showed that test conditions can be altered successfully provided the new conditions are performed consistently.

A new standard curve was prepared in the mill laboratory using standard durum wheat samples provided by the Wheat Research Institute. The standard sound durum wheat was a Tara cultivar from the 1987 harvest which represented an average PST value (0.351, Fig. 1b) for all the sound durum wheat samples listed in Materials and Methods. The standard 25, 50, 75, and 100% heat-damaged durum wheat samples were blends of the sound durum wheat with totally heat-damaged durum wheat from the same source. The sound standard was analysed in the commercial laboratory each day that commercial lines of durum wheat were being tested at wheat intake over a period of 6 months. The mean absorbance value for 36 determinations (range 1.240–0.892) was 1.047 and the standard deviation was 0.088. The range of PST values for the 272 artificially dried durum wheat lines tested was 0.811–1.676. None of these lines was considered heat-damaged when compared to the standard sound durum wheat value determined on the same day. Also, no problems in milling, dough-mixing, or extrusion were encountered when these lines were used for the manufacture of pasta products.

This result was fortunate for the manufacturer, but, to verify the PST, it would have been useful to detect one or more commercially heat-damaged durum wheat samples. It was possible, however, that the commercial grain driers took more care in their drying procedures with the knowledge that the mill was routinely testing for heat damage.

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Identification and discrimination of New Zealand bread wheat, durum wheat, and rye cultivars by RP-HPLC

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Abstract This paper describes modifications to the procedure for identification of New Zealand bread wheat, New Zealand and Australian durum wheat, and New Zealand and overseas rye cultivars by reversed-phase high-performance liquid chromatography (RP-HPLC) of the alcohol-soluble proteins (gliadins or secalins). Twenty-three outclassed and current bread wheat cultivars, six durum wheat cultivars, and four rye cultivars were examined. Each was found to be uniquely identifiable from its protein elution pattern. Rye had one peak that was clearly distinguished from all bread and durum wheats examined. This enabled the identification of rye in a wheat/rye mix. It may be possible, using this technique, for the proportion of rye in a mixture to be quantitated.

Keywords bread wheat; durum wheat; rye; gliadin; secalin; cultivar identification; RP-HPLC

INTRODUCTION

The identification of wheat varieties by reversed-phase high-performance liquid chromatography (RP-HPLC) of the gliadin storage proteins has been well documented both overseas and in New Zealand (Bietz et al. 1984; Bietz & Cobb 1985; Cressey 1987; Marchylo et al. 1988). This method is rapidly gaining acceptance as a more rapid and convenient method of identifying wheat cultivars than electrophoresis, particularly when rapid results are required or the total number of samples to be analysed is small (Wrigley et al. 1989). In addition, closely related cultivars which are difficult to distinguish by electrophoresis may be differentiated by RP-HPLC. This paper describes modifications made to the method of Cressey (1987) which have greatly improved the resolution of the RP-HPLC technique, allowing bread wheats with similar gliadin RP-HPLC patterns, such as 'Otane' and 'Oroua', to be more readily distinguished. A database of all New Zealand bread and durum wheats, major Australian durum wheats, and a selection of New Zealand and overseas rye cultivars has been compiled.

MATERIALS AND METHODS

Wheat and rye

All single cultivar samples analysed were either supplied by Crop Research Division, Department of Scientific and Industrial Research, Lincoln, New Zealand or were drawn from the 1987/88 Wheat Research Institute harvest evaluation file. Commercial millers and bakers supplied mixed grists. The following cereal cultivars were examined: 23 bread wheats currently or previously grown in New Zealand ('Abele', 'Advantage', 'Aotea', 'Arawa', 'Batten', 'Bounty', 'Crossbow', 'Haydock', 'Hilgendorf', 'Hotspur', 'Karamu', 'Kokart', 'Konini', 'Kopara', 'Kotare', 'Newbury', 'Otane', 'Oroua', 'Pegasus', 'Rongotea', 'Takahe', 'Tiritea', and 'Weka'); six current and outclassed durum wheats of New Zealand or Australian origin ('Aldura', 'Kamillaroi' (Aust), 'Tara', 'Vernum', 'Waitohi', and 'Yallaroi' (Aust)); and four rye cultivars of New Zealand, overseas, or wild origin ('Amino Dominant' (Europe), 'Rahu', 'Secale Montanum' (wild), and 'Snoopy Dwarf' (CIMMYT (International Maize and Wheat Improvement Centre)). The term "bread wheat" is used where a cultivar has been, or is currently, used for bread production. Some older wheat varieties are now

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used for other baked products. Table 1 presents bake score data for cultivars determined during the 1985 and 1988 harvests. These harvests were chosen because they are representative of typical weather conditions, neither boosting or lowering cultivar performance. Bake scores were determined using the WRI 50 g MDD minibake described by Swallow & Baruch (1986).

Sample preparation

Flour and meal samples were prepared by Brabender Quadrumat Junior, and Falling Number 3100 mills respectively. Flour (100 mg), or meal (200 mg), samples were extracted in capped 15 ml corex centrifuge tubes with aqueous ethanol (2 ml, 70% v/v) for 30 min, with occasional agitation on a vortex mixer. All extractions were performed at room temperature. Samples were centrifuged (48 000*g*, 15 min) and the clear supernatants removed for analysis.

Chromatographic conditions

Chromatography was performed using a Waters 600 solvent delivery/control system with a Waters WISP 712 automatic sample injector and a Waters 481 variable wavelength ultra violet detector. The column used was a Brownlee Aquapore RP-300 (220 × 4.6 mm) fitted with a Brownlee Aquapore RP-18 Newguard column (18 × 3.5 mm). Eluted components were detected at 210 nm and chromatographic traces recorded using the Waters Baseline 810 software operating on a personal computer. Solvents used for elution were: (A) water containing 0.06% (v/v) trifluoroacetic acid (TFA); and (B) acetonitrile containing 0.05% (v/v) TFA. Degassing was achieved by vacuum filtration through a 0.22 μm filter, rapid sparging with helium (100 ml/min, 10 min) and constant, slow helium sparging into capped, vented solvent reservoirs (10 ml/min).

Samples (20 μl) were injected onto the column which was maintained at 70°C using a Waters column heater. All samples were analysed in duplicate. A linear 48 min solvent gradient from 24 to 48% B with a 12 min hold at final concentration was used. The column was returned to the initial solvent composition over 1 min and allowed to re-equilibrate for 30 min before the next analysis. Flow rate was 0.5 ml/min for all analyses. It has been suggested by Marchylo & Kruger (1988) that injection of volumes larger than 5 μlitres causes misleading results because of protein washing off the column with the injection peak. However, the same authors have shown that when 70% ethanol is used as the extractant, this is only a severe problem for the ω-gliadins when the injection volume reaches 20 μlitres. As the peaks due to the ω-gliadins are not used in the identification process, and other gliadin fractions are largely unaffected at this injection volume, a multiple injection technique was considered undesirable as it precluded the use of an autosampler.

RESULTS AND DISCUSSION

Since the previous paper detailing New Zealand bread wheat cultivar identification using gliadin chromatograms (Cressey 1987), improvements have been made to the chromatographic conditions. The most significant of these has been the elevation of the column temperature from ambient to 70°C increasing the number of peaks observed, either increased resolution or changed column/protein as a result of specificity, and minimising variation in peak retention times (Bietz & Cobb 1985; Marchylo et al. 1988; S. M. Rutledge unpubl. data). The effect of elevating column temperature and modifying gradient conditions is shown in Fig. 1A–D. This has

Table 1 New Zealand bread wheat cultivars ranked by bake score.

Cultivar	Bake score[a]
Otane	25.5[b]
Kotare	24.1[b]
Arawa	23.7[c]
Oroua	23.6[c]
Weka	21.8[b]
Rongotea	21.6[c]
Konini	21.1[b]
Advantage	19.6[c]
Bounty	19.4[c]
Tiritea	18.2[c]
Kopara	17.6[c]
Takahe	17.2[c]
Crossbow	15.9[b]
Kokart	15.0[b]
Karamu	14.7[c]

[a] WRI 50 g MDD minibake bake scores determined as described by Swallow & Baruch (1986).

[b] 1988 data.

[c] 1985 data. These years were chosen because they represent typical harvests with no strong seasonal swings. Samples suffering weather damage have been excluded from calculations. The following cultivars have not been grown in sufficient quantity since 1984 to enable an accurate ranking: 'Abele'; 'Aotea'; 'Batten' (due for release); 'Haydock'; 'Hilgendorf'; 'Hotspur'; 'Newbury'; and 'Pegasus'.

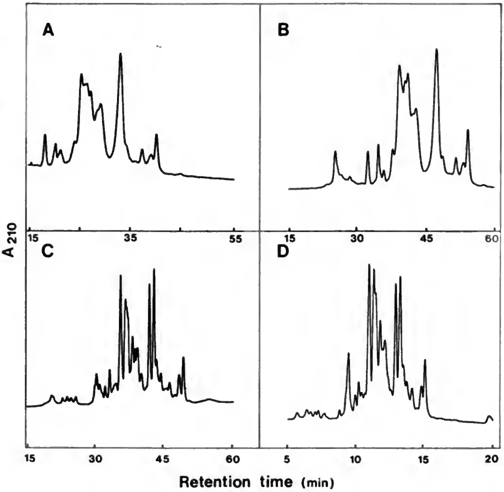


Fig. 1 RP-HPLC chromatograms of wheat gliadins cv. Otane on a Brownlee Aquapore RP-300 column (220 × 4.6 mm). Gliadins were extracted as described in the text. Protein elution schemes were: **A,** linear gradient from 28 to 50% acetonitrile in 55 min at 1 ml/min and 20°C (as per Cressey 1987); **B,** linear gradient from 24 to 48% acetonitrile in 48 min + 12 min hold at final concentration at 0.5 ml/min and 20°C; **C,** as for B but with column temperature increased to 70°C; **D,** linear gradient from 24 to 48% acetonitrile in 15 min + 5 min hold at final concentration at 2 ml/min and 70°C (S. M. Rutledge, unpubl. data). Solvent peaks are not shown.

led to an expansion of ongoing bread wheat cultivar identification to include durum wheat and rye cultivars.

Bread and durum wheats

Bread and durum wheat chromatograms typically contain 25 major and minor components. Fig. 2A–F illustrate the chromatograms of several common bread wheat cultivars. Comparison of Fig. 2 with Fig. 3A–D shows that durum wheat chromatograms are similar to, although distinguishable from, those of bread wheat. All durum wheats analysed have major peaks at retention times of c. 35.2, 38.4, 43.2, and 48.2 min. These are marked * on Fig. 3A. The peak at 35.2 min is also a major component in the bread wheats 'Rongotea', 'Otane', and 'Oroua' (first marked peak, Fig. 2A), as is the peak at 48.2 min in 'Rongotea' only. Major peaks found in bread wheats that do not appear to any great extent in durums occur at 36.4, 41.7, and 42.8 min (marked peaks, Fig. 2A).

Visual inspection of the chromatograms of unknown wheats and comparison of peak presence/absence and relative peak heights with those of library chromatograms is usually sufficient for cultivar identification. Identification is then confirmed by running an authentic sample of the assumed cultivar. For bread wheats, the greatest variation in chromatogram appearance occurs between 35 and 40 min, and to a lesser extent between 47 and 49 min. Likewise durum wheat variation occurs largely between 35 and 40 min, with fewer variations between 40 and 45 min.

Rye

To enable direct comparison of rye and wheat chromatograms the same elution gradient was used. Fig. 3E, F shows that rye-secalin chromatograms are easily distinguished from wheat gliadins by grouping most peaks into three regions. These range from c. 27.9 to 28.4, 39.2 to 40.5, and 44.5 to 45.8 min. In addition, a peak of moderate size (often displaying small, poorly resolved satellite peaks) elutes at 50.2 min. Analysis of a 1:1 ratio of 'Rahu':'Otane' showed that this rye peak was clearly distinguishable from the latest eluting wheat peak (48.2 min). If it is possible to accurately quantify this peak in wheat/rye mixtures then it may be that RP-HPLC could be used as a quantitative analysis tool for such mixtures. In general, differences between rye cultivars are less

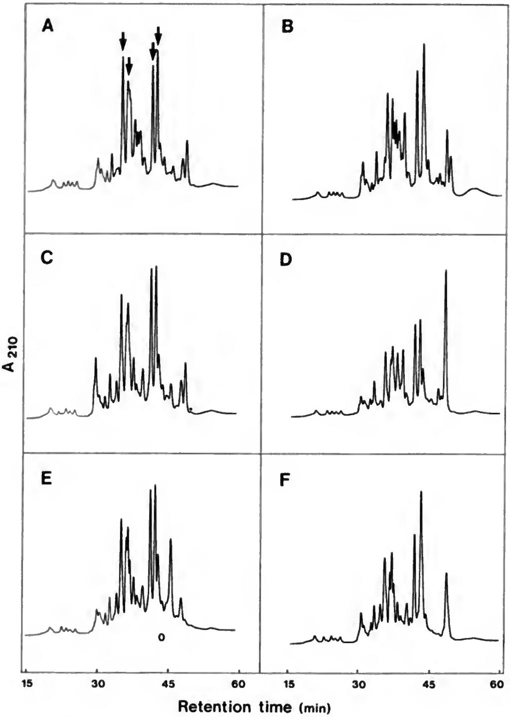


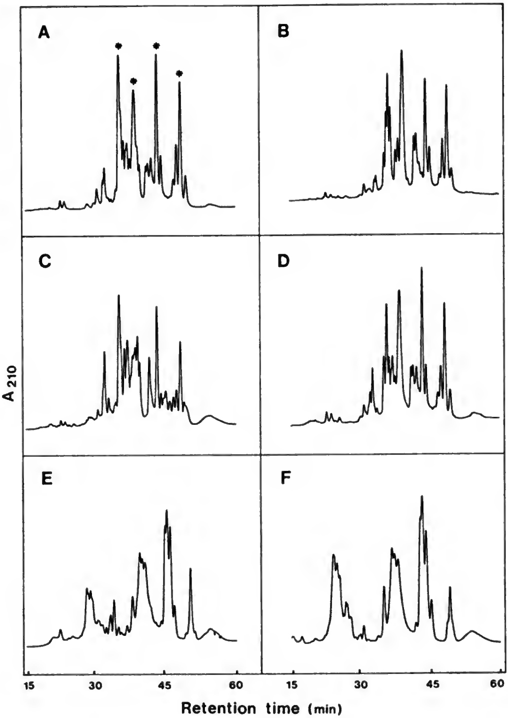
Fig. 2 RP-HPLC chromatograms of bread wheat gliadins on a Brownlee Aquapore RP-300 column (220 × 4.6 mm). Gradient conditions were 24–48% acetonitrile over 48 min + 12 min hold at final concentration at 0.5 ml/min and 70°C. Cultivars illustrated are: A, 'Otane'; B, 'Kotare'; C, 'Oroua'; D, 'Rongotea'; E, 'Karamu'; and F, 'Konini'. Peaks marked with an arrow (↓) elute at 35.2, 36.4, 41.7, and 42.8 min respectively. Solvent peaks are not shown.

pronounced than those found between wheat cultivars, although not sufficient to inhibit cultivar identification. It is our experience that the most distinguishing region among rye cultivars is for peaks eluted between 48 and 52 min.

Variability of chromatography

Two sources of variation were quantified: (a) variation between injections of the same sample on the same day; and (b) variation between different samples of the same material on different days. Additionally, it was noted that when a column from a different batch was used, retention times varied by c. 7%, together with changes in column specificity, causing slight differences in chromatogram appearance. Samples of cultivar Otane analysed on the column used for the reproducibility checks had major peaks with retention times of c. 37.0 and 44.5, and a single peak with a shoulder at 52.7 min, corresponding to peaks in Fig. 2A at 35.2, 41.7, and the doublet at c. 48.8 min.

Using the three major peaks at 37.0, 44.5, and 52.7 min mentioned above, coefficients of variation were calculated using the method of Sokal & Rohlf (1981). For Source (a) these were 0.44, 0.52, and 0.89% (peak area) respectively, and 0.12, 0.12, and 0.05% (retention time) respectively ($n=9$). Coefficients of variation for Source (b) were 2.24, 2.14,



.3 RP-HPLC chromatograms lurum wheat gliadins and rye alins on a Brownlee Aquapore ·300 column (220 × 4.6 mm). idient conditions were 24–48% tonitrile over 48 min + 12 min d at final concentration, at ml/min, and 70°C. Cultivars strated are durum wheats: A, allaroi'; B, 'Kamillaroi'; C, aitohi'; and D, 'Tara'; and s—E, 'Rahu'; F, 'Amino minant'. Durum wheat peaks rked (*) elute at 35.2, 38.4, 43.2, 1 48.2 min respectively. Solvent aks are not shown.

nd 0.96% (peak area) respectively, and 0.61, 0.70, nd 0.52% (retention time) respectively (n=18). nterestingly, the coefficients of variation for peaks vhich are not fully resolved (37.0 and 44.5 min) are onsiderably larger than the peak at 52.7 which has aseline resolution. This suggests that a large proporion of the day-to-day variation observed in peak rea is caused by fine differences in the integration f results, rather than the variability of the technique itself. However, long-term variation as a result of seasonal effects or the use of different column batches, is likely to have a more profound effect on chromatogram appearance and final identification.

CONCLUSIONS

RP-HPLC of 70% alcohol-extractable cereal proteins is now an accepted method of cultivar identification. Modifications made to previously published methods have increased the resolution obtained. It is possible to uniquely identify all bread wheat, durum wheat, and rye cultivars currently grown in New Zealand. All of the rye cultivars examined had one well-separated peak which eluted later than the latest eluting wheat peak. It is possible that this feature can be used to quantify the amount of rye present in mixtures of rye and wheat, such as the kibble used in multigrain bread.

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Effects of fumigation, cold storage, and fungicide treatment of planting cloves on yield and quality of garlic

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Abstract Fumigation with methyl bromide at 32 g/m^3 for 2 h at 21°C has been recommended for the control of the wheat curl mite (*Aceria tulipae*) in stored garlic. The effects of fumigation 68 days before planting, of cold storage at 4.5°C for 30 or 60 days before planting, and of fungicide treatment of planting cloves were investigated in healthy mite-free 'Marlborough White' garlic planted at Crop Research Division, DSIR, Lincoln, Canterbury on 17 May and 15 June 1983. Fumigation with or without cold storage decreased plant populations, mean bulb weights, and yields, and increased percentages of rough bulbs with side cloves. Cold storage for 60 days with or without fumigation decreased mean bulb weights and yields and increased percentages of rough bulbs with side cloves. The effect of cold storage for 60 days on percentages of rough bulbs was less severe with fumigation than without. Cold storage for 30 days had smaller effects. There were no phytotoxic or beneficial effects of captan, benomyl, or vinclozolin treatment of planting cloves.

Keywords *Allium sativum*; garlic; 'Marlborough White'; fumigation; cold storage; fungicides; emergence; mortality; yield; side cloves

INTRODUCTION

Heavy infestations of the wheat curl mite (*Aceria tulipae*) in stored garlic have been recorded in Marlborough, the main garlic growing area in New Zealand (Manson 1970). In California, the wheat curl mite has caused considerable losses in stored garlic and leaf distortion in young garlic plants (Sims et al. 1976). Fumigation of garlic bulbs with methyl bromide, at the rate of 32 g/m^3 for 2 h at 21°C, has been recommended for the control of mites (Jones & Mann 1963). It was also a required condition for the importation to the United States of garlic infected with larval stages of weevils (*Brachycerus* sps) or of a carpenter worm moth (*Despessa ulula*) (Roth & Richardson 1963). Roth & Richardson (1963) investigated the effects of fumigation on quality of stored garlic. They showed that tolerance to fumigation was excellent when applied at atmospheric pressure and was only slightly affected when applied under vacuum at a pressure of c. 1/12 bar. However, it is possible that the toxicity of methyl bromide would delay sprouting and subsequently reduce plant growth and yield. Also, delayed sprouting could increase the incidence of blue mould (*Penicillium* sps) which would reduce plant population and yield (Mann & Lewis 1956).

In California, 'Californian Late' garlic sprouted more rapidly after storage at 5°C than at 10°, 15°, 0°, and 20°C, in decreasing order (Mann & Lewis 1956). The possibility was considered that cold storage would counteract an expected delay in sprouting resulting from fumigation, thereby reducing the incidence of blue mould.

At Lincoln, crop losses from blue mould, Fusarium rot (*Fusarium* sps), and dry rot (*Botryotinia porri*) have occurred in garlic grown from apparently symptomless planting cloves on land that had not been previously used for garlic production (J. Lammerink unpubl. data). Fungicide treatment of planting cloves was suggested in an attempt to control these diseases (I.C. Harvey pers. comm.).

For these reasons, two trials were conducted at Lincoln to investigate the effects of fumigation, cold

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storage, and fungicide treatment of mite-free, healthy-looking planting stock on plant emergence, mean bulb weight, yield, and quality of garlic under New Zealand conditions.

MATERIALS AND METHODS

Both trials were of the same split-plot design with four replicates. Each trial was arranged as a factorial of fumigation and cold storage treatments of bulbs of 'Marlborough White' garlic with fungicide sub-treatments of the planting cloves.

There were two levels of fumigation: nil and methyl bromide at 32 g/m^3 for 2 h at 21°C, applied 68 days before planting. Fumigation was carried out under vacuum at 1/15 bar pressure in the first trial and at atmospheric pressure (c. 1 bar) in the second trial.

There were three levels of cold storage: nil, 30, and 60 days at 4.5 ± 1°C. Cold storage commenced on either 12 March or 11 April in the first trial and on either 8 April or 8 May in the second trial. When not in cold storage, the bulbs were kept in an open barn with mean daily maximum and minimum temperatures that were c. 1°C (SE = 0.5) lower and higher respectively than the mean screen temperatures presented in Table 1. No attempts were made to control relative humidity during storage.

The fungicide sub-treatments were: nil, captan, benomyl, and vinclozolin. They were applied to healthy-looking cloves as dry powders at the rate of 10 g/kg of cloves. The cloves were then moistened and subsequently dried, 2 days before planting. The mean clove weights per subplot varied from 3.0 to 3.4 g.

The trials were planted adjacent to each other on 17 May and 15 June 1983. They were located at Crop Research Division, DSIR, Lincoln, on a Templeton silt loam after a wheat crop. Each plot consisted of a single row 4.5 m long. Rows were 0.5 m apart and plants were spaced 0.1 m in the row. Reverted superphosphate at 500 kg/ha, urea at 200 kg/ha, and the herbicide trifluralin (40% a.i.) at 2 litre/ha were incorporated in the soil 3 weeks before planting. Further weed control was by hand-hoeing. The trials received one sprinkler irrigation on 18 November following an application of urea at 100 kg/ha.

Plant counts were made on 27 July, 15 August, and 19 January. All plants were lifted for harvesting on 19 January when they had started to lodge and the foliage was turning yellow. After 6 days drying in the field, the plants were stored for 6 weeks in an open barn. The bulbs were then topped, tailed, weighed, and sorted into two grades (Fig. 1):

> "Marketable"—nil to three side cloves; smooth after removal of side cloves.
>
> "Unmarketable"—four to many side cloves; rough. (Fig. 1).

The number of marketable bulbs as a percentage of the number of bulbs harvested was calculated for each plot. Analysis of variance was carried out on plant numbers, mean bulb weight, and yield, and on percentage of marketable bulbs after angular transformation.

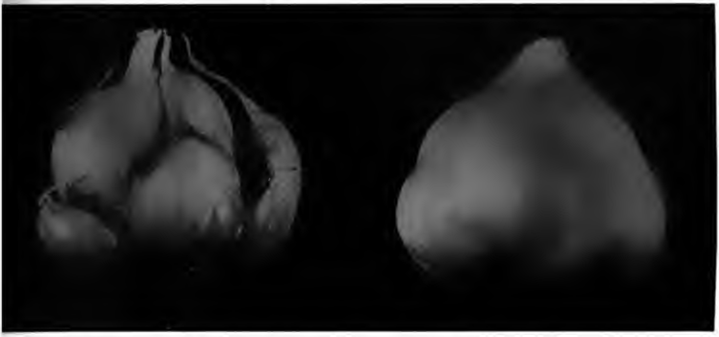
RESULTS AND DISCUSSION

Fungicides

The fungicide sub-treatments and their interactions with cold storage and fumigation had no significant effects on plant numbers, mean bulb weight, yield, or percentage of marketable bulbs in either trial. In both trials, they contributed less than 7% to the total variability of each of these characters. No fungal diseases were detected after plant emergence.

Table 1 Daily maximum, minimum, and mean screen temperatures at Lincoln. Means are for periods of cold treatment during storage of garlic before planting on 17 May and 15 June 1983.

Planting date	Period	Daily max. Mean	Daily max. SE	Daily min. Mean	Daily min. SE	Daily mean Mean	Daily mean SE
Trial 1							
17 May	12 Mar–10 Apr	20.2	0.96	7.5	0.68	13.9	0.79
	11 Apr–10 May	16.3	0.76	6.7	0.62	11.4	0.46
Trial 2							
15 Jun	8 Apr–7 May	15.9	0.79	6.4	0.60	11.1	0.48
	8 May–6 Jun	12.1	0.63	3.4	0.62	7.8	0.50



. 1 'Marlborough White' garlic: "marketable" (left) and "unmarketable" (right) grades.

Table 2 'Marlborough White' garlic, with or without fumigation and with cold treatment for 0, 30, or 60 days, planted on 17 May and 15 June 1983. Numbers of plants at different dates as percentage of number of cloves planted, mean weights of bulb, total bulb yields, and percentage of marketable bulbs.

Cold treatment	Non-fumigated			Fumigated				
(days)	0	30	60	0	30	60	Int[3]	LSD$_{0.05}$
Trial 1								
Planting date: 17 May								
Plant number (%)[1]								
27 Jul	64	60	73	88	83	25	***	6.3
15 Aug	97	96	98	96	95	70	***	5.1
19 Jan	100	100	98	95	94	84	**	3.8
Bulb weight (g)	62.3	55.5	48.2	55.9	48.5	47.5	*	3.83
Bulb yield (t/ha)	12.5	11.1	9.4	10.7	8.7	7.9	NS	0.88
Marketable (%)[2]	95.6	83.1	67.0	89.7	85.2	87.1	–	–
Angular transf.	84.4	67.7	55.7	74.3	69.6	71.4	***	8.50
Trial 2								
Planting date: 15 Jun								
Plant number (%)[1]								
27 Jul	58	57	36	21	16	4	NS	8.0
15 Aug	98	94	92	85	81	58	***	4.7
19 Jan	100	99	99	91	92	90	NS	3.1
Bulb weight (g)	62.0	61.5	52.5	53.5	51.9	47.1	NS	4.86
Bulb yield (t/ha)	12.6	12.3	10.3	9.9	9.7	8.6	NS	1.00
Marketable (%)[2]	96.9	94.6	47.1	88.8	89.7	74.1	–	–
Angular transf.	86.6	80.4	43.2	72.7	73.7	60.3	***	7.56

[1] As percentage of number of cloves planted.

[2] As percentage of number of bulbs harvested.

[3] Significance of fumigation × cold treatment interaction; * = $P < 0.05$; ** = $P < 0.01$; *** = $P = 0.001$.

Cold storage for 60 days without fumigation

In the first trial, cold storage for 60 days caused more plant emergence by 27 July, 71 days after planting, but it had no effect on plant numbers by 15 August (Table 2). However, in the second trial, planted on 15 June, this treatment had a retarding effect on emergence by 27 July and this effect was still just significant on 15 August, 61 days after planting. Eventually, plant emergence in both trials was virtually complete by harvest on 19 January.

After planting, the mean earth temperatures at 10 cm depth were 4.5°C (SE = 0.39), 4.6°C (SE = 0.32), and 4.6°C (SE = 0.50) over the periods from 17 May to 14 June, 15 June to 26 July, and 27 July to 15 August respectively. These temperatures provided sufficient cold to induce sprouting and subsequent bulbing.

Mean bulb weight and yield were reduced 23 and 25% respecitvely in the first trial and 15 and 18% respectively in the second trial by cold storage for 60 days. The percentage of marketable bulbs was also reduced by this treatment: by 30% in the first trial and by 51% in the second trial. These results confirm observations by Mann & Minges (1958) that cold storage increased the incidence of rough bulbs with side cloves.

Fumigation without cold storage

Plant emergence by 27 July was enhanced by fumigation in the first trial, but retarded in the second trial (Table 2). It caused 5% mortality in the first trial and 9% mortality in the second trial, as indicated by numbers of plants at harvest on 19 January.

Fumigation decreased mean bulb weight, yield, and percentage of marketable bulbs by 10, 14, and 6% in the first trial and 14, 21, and 8% in the second trial. Bulb weight did not compensate for the reduced number of surviving plants.

Fumigation with cold storage for 60 days

The negative effects of fumigation on emergence, plant numbers, bulb weight, and yield increased in both trials and the negative effect on percentage of marketable bulbs increased in the second trial only, when it was combined with cold storage for 60 days. However, in both trials the percentage of marketable bulbs was higher after cold storage for 60 days with fumigation than after cold storage alone.

Cold storage for 30 days

Cold storage for 30 days, whether combined with fumigation or not, had no effect on emergence and plant numbers in either trial. However, it did reduce mean bulb weight, yield, and percentage of marketable bulbs in the first trial, but not in the second trial. The lesser response to cold storage for 30 days in the second trial was expected because the temperature was only 3.3°C lower than the mean of the mean daily ambient temperatures, compared with 6.9°C in the first trial.

Comparison between the two trials

Both fumigation and cold storage for 60 days had different effects on plant emergence as recorded on 27 July in the two trials. Emergence was enhanced by fumigation without cold storage and by cold storage without fumigation in the first trial, but delayed in the second trial. The interaction between the fumigation and cold storage factors was highly significant in the first trial, but non-significant in the second trial. Except for these effects on emergence, results of the two trials were similar despite differences in fumigation conditions and planting times.

CONCLUSION

Results from this experiment highlight the phytotoxic impact of fumigation of garlic, expressed as a reduction in plant numbers (5–9%), mean bulb weight (10–14%), yield (14–21%), and percentage of marketable bulbs (6–8%). This impact was not counteracted by cold storage at 4.5°C or by fungicide treatment of the planting cloves. The experiment was conducted with mite-free garlic. However, it is speculated that if a serious infestation of the wheat curl mite were present, the negative effects of fumigation would probably be more than compensated by the beneficial effects of controlling the infestation.

Cold storage had detrimental effects on yield and quality of garlic, and no apparent benefits. Treatment of planting cloves with captan, benomyl, or vinclozolin had no phytotoxic effects. Treatment may be worthwhile if fungal diseases are known to be present.

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ultivar release

hau Early' red raspberry (*Rubus idaeus* L.)

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bstract 'Ohau Early' red raspberry (*Rubus idaeus* .) is a new cultivar resulting from the cross 'Lloyd eorge' × 'Festival'. Evaluated for three seasons at evin, it consistently produced high yields of dark ed, firm, rot-resistant fruit. It had a short harvest eriod (6–12 days), early in the season. It appears to e a particularly suitable cultivar for machine-arvesting.

eywords 'Ohau Early'; red raspberry; *Rubus daeus* L.; harvest period; machine-harvesting; ultivar

INTRODUCTION

Red raspberries (*Rubus idaeus* L.) are a cool temperate crop grown worldwide. In New Zealand, much of our red raspberry production is individually quick frozen (IQF) or bulk frozen and sold to processors locally and overseas. Fruit for processing can be machine-harvested, an option being explored by growers as hand-picking costs rise and returns for fruit remain relatively low. For this reason, a red raspberry selection programme, aimed at developing good machine-harvestable cultivars, was initiated at Levin Horticultural Research Centre (LHRC) in 1968. 'Ohau Early' is a cultivar arising from this programme.

ORIGIN

'Ohau Early' is a seedling selected from the progeny of a 'Lloyd George' × 'Festival' cross. It, and 780 seedlings from a number of different crosses, were planted in the field in 1979. Plants were observed regularly and unsuitable seedlings rogued over 3 years. Characteristics selected for (in no particular order) were strong fruiting laterals, ease of fruit removal from the plug, high relative yield, fruit firmness, and a short fruiting period. During 1982/83, 'Ohau Early' and four other promising selections were both vegetatively and micropropagated, then planted in a replicated trial in the winter of 1984. Fruiting characteristics were evaluated in the three subsequent seasons (1985–87) by the machine-harvesting and recording of fruit weights obtained, and regular laboratory analysis of representative fruit samples.

'Ohau Early' has been registered with the New Zealand Plant Varieties Office (Plant Selector's Rights No. 313).

DESCRIPTION

'Ohau Early' is a vigorous, early flowering and fruiting red raspberry cultivar with a harvest period which consistently starts 9 days before 'Skeena'. Fruit are dark red in colour, small (mean berry weight is 2.24 g), and rounded in shape. With firmness comparable to 'Skeena', they detach relatively easily from the plug and have only a slight tendency to crumble. Harvest period is of short to medium length: harvest could be completed within 6–12 days during the three seasons in which we evaluated this cultivar.

Plants produce a large number of new canes averaging, after winter pruning and training, 16 per metre of row which is a similar cane density to that produced by 'Skeena'. 'Ohau Early' canes do not tend to branch and produce short laterals which resist damage by wind or machine-harvester.

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Table 1 Saleable, reject, and diseased fruit yields for the red raspberry cultivars 'Skeena' and 'Ohau Early' during three consecutive seasons. NS = not significant.

	1985			1986			1987		
	Skeena	Ohau Early	$LSD_{0.05}$	Skeena	Ohau Early	$LSD_{0.05}$	Skeena	Ohau Early	$LSD_{0.05}$
Saleable yield (t/ha)	6.59	9.06	0.10	1.85	5.09	2.91	4.94	8.23	2.99
Reject yield (t/ha)	2.98	0.42	NS	0.44	0.14	0.25	0.97	0.34	0.58
Diseased yield (t/ha)	1.84	0.26	NS	0.08	0.23	0.11	0.56	0.31	0.23

Table 2 Saleable, reject, and diseased yields, expressed as a percentage of total recorded yield, for the red raspberry cultivars 'Skeena' and 'Ohau Early'. Rainfall figures for December, when fruit was harvested, are shown for reference.

	1985		1986		1987	
	Skeena	Ohau Early	Skeena	Ohau Early	Skeena	Ohau Early
Saleable yield	58	93	78	93	76	92.5
Reject yield	26	4	18.5	3	15	4
Diseased yield	16	3	3.5	4	9	3.5
December rainfall (mm)	164.5		27.4		97.1	

PERFORMANCE

Performance data were collected, during three seasons, from a trial area established at LHRC in 1984. All plots were machine-harvested, using a fingerdrum harvester, at 3-day intervals starting just after first fruit fall. The most appropriate machine settings for each cultivar or selection were determined by trial on extra rows set aside for this purpose. Yields of saleable, reject (green), and diseased fruit were recorded. 'Ohau Early' always produced significantly greater saleable yields than 'Skeena' and smaller amounts of reject fruit, significant in the 1986 and 1987 seasons, despite it's higher saleable yield (Table 1).

The amount of diseased fruit removed from 'Ohau Early' plots, as a proportion of total yield, tended to be lower than that for Skeena in the 1985 and 1987 seasons (Table 2). In the 1986 season, both cultivars had similar proportions of diseased fruit, possibly reflecting the low rainfall occurring during this harvest season (Table 2). In all seasons, the major disease present was grey mould (*Botrytis cinerea*).

The weighted mean berry weight of 'Ohau Early' over three seasons (using individual harvest yields as a variate) was significantly smaller than that of 'Skeena' (2.24 g and 3.43 g respectively).

Laboratory analyses for colour intensity, fruit firmness, and titratable acidity were performed in all three seasons: methods are described in O'Donoghue & Martin (1988). Sugar levels were assessed in the 1986 and 1987 seasons only. In the 1986 season, sugars were extracted from freeze-dried raspberry tissue with 62.5% methanol and separated by High Pressure Liquid Chromatography using a Dextropak column. Results are quoted as grams (mono- and disaccharides) per 100 g of fresh fruit. In the 1987 season, sugar levels were measured using a hand-held Atago refractometer; results are expressed in Brix.

There was no consistent relationship between fruit firmness of the two cultivars over three seasons (Table 3). Colour intensity of juice extracted from

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Table 3 Fruit quality characteristics of red raspberry cultivars Skeena and Ohau Early during three consecutive seasons. All figures are weighted means (using individual harvest yields as a variate) for the season.

	1985			1986			1987		
	Skeena	Ohau Early	$LSD_{0.05}$	Skeena	Ohau Early	$LSD_{0.05}$	Skeena	Ohau Early	$LSD_{0.05}$
Firmness (% juice loss)	35.3	25.2	2.8	25.9	24.1	NS	23.4	30.2	3.8
Colour (AU[1])	16.7	37.2	3.2	18.8	47.5	4.3	19.2	36.4	2.2
Acidity ($MeqH^+$/g fruit)	0.254	0.238	NS	0.264	0.325	0.026	0.235	0.275	0.008
Sugar content (g/100 g fresh wt)	–	–	–	5.97	4.38	0.36	–	–	–
(°Brix)	–	–	–	–	–	–	8.28	5.92	0.33

[1] Absorbance units of undiluted juice

'Ohau Early' raspberry pulp was always significantly higher than that of juice from 'Skeena' pulp (Table 3). 'Ohau Early' fruit were generally more acidic and had lower sugar levels than 'Skeena' fruit; these differences were significant in the 1986 and 1987 seasons (Table 3).

A compact harvest period and high efficiency with which the berry can be removed from the plant are attributes which make 'Ohau Early' a promising new raspberry cultivar for machine-harvesting, extending machine use over a longer period of time.

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Plant disease record

Vew plant disease records in New Zealand: niscellaneous fungal pathogens II*

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Abstract Ten fungi, *Cladosporium colocasiae* Sawada (on *Colocasia esculenta* (L.) Schott), *Colletotrichum malvarum* (Braun et Casp.) Southworth (on *Lavatera* sp.), *Frommeella duchesneae* (Arthur) Yohem, Cummins et Gilbertson (on *Duchesnea indica* (Andrews) Focke), *Hyalopsora polypodii* (Diet.) Magnus (on *Deparia petersenii* (Kunze) Kato ssp. *congrua* (Brackenr.) Kato), *Mycocentrospora cladosporioides* (Sacc.) P. Costa ex Deighton (on *Olea europaea* L.), *Phaeoramularia eupatorii-odorati* (Yen) Liu et Guo (on *Ageratina adenophora* (Spreng.) King et Robinson), *Phyllachora bulbosa* Parbery (on *Zoysia pauciflora* Mez and *Z. planifolia* Zotov), *P. setariicola* Speg. (on *Oplismenus hirtellus* (L.) Beauv.), *Puccinia festucae* Plowr. (on *Festuca rubra* L.), and *Tolyposporium rodwayi* McAlp. (on *Lepidosperma laterale* R. Br.) are recorded in New Zealand for the first time; 35 new host/pathogen records are given for 31 other species. Two existing records, *Ramularia sphaeroidea* Sacc. on *Lotus corniculatus* L. and *Ustilago agropyri* McAlp. on *Anthoxanthum odoratum* L. are deleted following redetermination of the hosts as *L. pedunculatus* Cav. and *Rytidosperma* sp. respectively. Two other records, *Cercospora eupatorii* Peck on *Eupatorium adenophorum* Spreng. (≡ *Ageratina adenophora*) and *Phyllachora punctum* (Schwein.) Orton on *Oplismenus hirtellus* are deleted following redetermination of the fungi as *Phaeoramularia eupatorii-odorati* and *Phyllachora setariicola*, respectively.

Keywords new records; plant diseases; fungi; New Zealand

INTRODUCTION

Forty-six host/pathogen records are newly listed for New Zealand. Included amongst these combinations are 18 rusts, 4 smuts, 3 tar spots, 1 white rust, and several leaf spots and blotches. The hosts are mainly introduced weeds or indigenous plants of little economic importance.

Specimens of all the fungi are housed in the herbarium (PDD), DSIR Plant Protection (formerly Plant Diseases Division), Auckland, and for each specimen, the PDD accession number is given. Some fungi were extracted from plant specimens held in the herbarium of Botany Division, DSIR, Lincoln (CHR). For the rust fungi, II indicates the presence of uredinia, II^{a} amphispores, and III the presence of telia. Specimen localities are assigned to geographical areas as defined by Crosby et al. (1976). An asterisk indicates that the host is introduced.

FUNGI PREVIOUSLY UNRECORDED IN NEW ZEALAND

Cladosporium colocasiae Sawada, *Rep. nat. Hist. Assoc., Formosa 25:* 125, 1916

on **Colocasia esculenta* (L.) Schott (Araceae) Auckland, Leigh, 13.I.1974, C. Leach (PDD 32052); Epsom, 22.IV.1988, E. H. C. McKenzie (PDD 48693). Coromandel, Little Barrier Island, 6.IV.1988, E. H. C. McKenzie (PDD 48715).

Brown leaf spot, ghost spot, false spot, of older leaves. Reddish-brown, circular or irregular, diffuse blotches on either leaf surface, sometimes with a blackish, diffuse centre. Usually less evident on opposite surface of leaf. Sometimes surrounded by yellow halo, or with a dark-brown diffuse border. Spots up to 15 mm diam., much smaller when there are many spots on the one leaf. Colonies amphigenous. Conidiophores macronematous,

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mononematous, single, unbranched, straight or flexuous, smooth, septate, brown, paler towards the apex, up to 300 µm long × 3-6 µm thick. Conidiogenous cells polyblastic, integrated, terminal becoming inter-calary, with nodose swellings 4–8 µm wide, conidial scars on swellings thickened and slightly prominent; scar 1–2 µm wide. Conidia in branched or simple chains, cylindrical, usually narrower in the middle, rounded at the ends, pale brown, smooth, (0–)1(–2) septate, (11–)13–18(–22) µm long × (5–)6–8(–10) µm wide; hilum prominent, 1–2 µm thick.

This fungus is circumglobal in the tropics and is widespread on taro in the Pacific. David (1988) described and figured the fungus.

Colletotrichum malvarum (Braun et Casp.) Southworth, *J. mycol. 6*: 116, 1890
≡ *Steirochaete malvarum* Braun et Caspary, *Krankh. Pfl.*: 28, 1854

on **Lavatera* sp. 'Mont Blanc' (Malvaceae) Waikato, Hamilton, Gordonton Road, 24.X.1989, P.G. Broadhurst (PDD 56372).

Leaf spots round or irregular in shape, up to 5 mm diam., pale brown, diffuse at the margin. Numerous small, setose acervuli in central part of the spots. Stem lesions elliptic, extending up to 20 mm along stems and petioles. On potato dextrose agar, colonies slow-growing, from single conidia 5–8 mm diam. after 7 days. Acervuli pale, numerous setae, conidial ooze orange-brown, conidia cylindric, rounded apex, often tapering slightly to base, 12–15.5 × 4–5 µm. Colonies becoming very dark in reverse, aerial mycelium dense, tufted, pale- to dark-grey. No *Glomerella* (teliomorph) state formed.

Morphologically this fungus fits *Colletotrichum gloeosporioides* (Penzig) Penzig et Sacc., but it is confined to various Malvaceae on which it is associated with stem and leaf spotting. It is distinct culturally with its slow growth rate, dense aerial mycelium, and numerous setae.

Frommeella duchesneae (Arthur) Yohem, Cummins et Gilbertson, *Mycotaxon 22*: 452, 1985 (Fig. 1).
≡ *Kuehneola duchesneae* Arthur, *N. Am. Flora 7:* 185, 1912

on **Duchesnea indica* (Andrews) Focke (Rosaceae) Auckland, Tiritiri Matangi Island, 14.II.1988, P. K. Buchanan (PDD 48429) II; Mt Albert, 17.II.1988, E. H. C. McKenzie (PDD 48665) II, III; Henderson, 21.II.1988, P. J. Brook (PDD 48666) II, III. Coromandel, 7.IX.1989, E. H. C. McKenzie (PDD 56212) II; Bay of Plenty, Te Puke, 4.I.1989, S. R. Pennycook (PDD 55149) II.

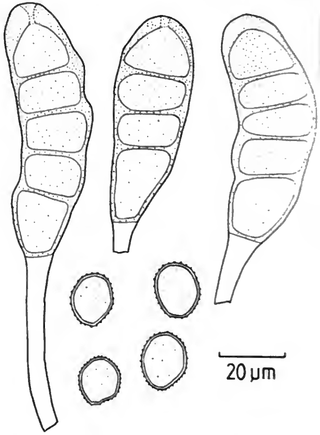
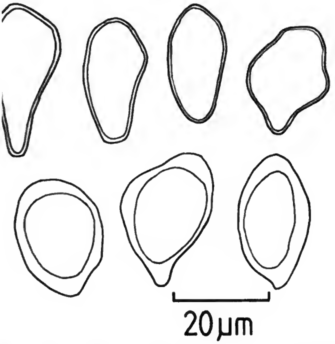


Fig. 1 Teliospores and urediniospores of *Frommeella duchesneae* (PDD 48665). Specimen mounted in hydrous lactophenol.

Rust. Uredinia hypophyllous, pulvinate, pulverulent, orange when fresh becoming yellow with age, with a few peripheral, colourless, thin-walled, cylindrical paraphyses 6.5–8 µm wide. Urediniospores (16–)17–19(–22) × (13–)14–16(–17) µm, globoid or broadly ellipsoid, wall 0.5–1 µm thick, pale, echinulate, germ pores obscure (3 and equatorial, Arthur 1912). Telia hypophyllous, arising from the same sori as the uredinia, pulverulent, cinnamon-brown. Teliospores 55–80 × 23–26 µm, cylindrical or clavate, straight or slightly curved, 3–7-celled, sometimes constricted at septa, wall 0.75–2.5 µm thick at sides, 4–6 µm at apex, smooth, golden-brown, sometimes paler basally; pedicels up to 65 × 5–7.5 µm, persistent, colourless.

This fungus, originally described from the United States, was found to be widespread in Auckland on *Duchesnea indica* in February 1988. At that time it was not present in the Bay of Plenty, where regular examination of *D. indica* first detected the rus



g. 2 Urediniospores and amphispores of *Hyalopsora lypodii* (PDD 54740). Specimen mounted in hydrous ctophenol.

proximately 1 year later, in January 1989 (S. R. nnycook and D. R. W. Watson pers. comm.).

yalopsora polypodii (Diet.) Magnus, *Ber. dt. bot. es. 19*: 582, 1901 (Fig. 2).

Pucciniastrum polypodii Dietel, *Hedwigia 38*: 50, 1899

n *Deparia petersenii* (Kunze) Kato ssp. *congrua* Brackenr.) Kato (Dryopteridaceae) Northland, angihua Reserve, 9.IV.1988, R. E. Beever (PDD 8698) II; Whangarei, Hutchinson's Reserve, 4.IX.1988, N. & T. Clements (PDD 54740) II, II*; lgunguru, Crawford Reserve, May 1988, N. & T. lements (PDD 48852) II.

ust. Uredinia amphigenous but mainly epiphyllous, ingle or grouped, sometimes on vein-limited, iscoloured areas, bullate, pulverulent, golden-ellow, circular or elongate. Urediniospores 20–)23–30(–32) × (11–)14–15(–16) µm, obovoid, llipsoid, or angular, wall 0.5(–1) µm thick, pale, mooth, germ pores obscure (2–4 and equatorial, liratsuka 1936). Amphispores 19–30 × 15–21 µm, bovoid, ellipsoid, or angular, wall 1.5–3.5 µm hick, pale, smooth, (germ pores 4–8, scattered, liratsuka 1936).

his rust occurs on several genera of ferns in the amily Dryopteridaceae in North America, Europe, nd Japan. Most species of *Hyalopsora* produce spermatia and aecia on *Abies* spp., but no alternate host is known for *H. polypodii*.

Mycocentrospora cladosporioides (Sacc.) P. Costa ex Deighton, *Mycol. Pap. 151*: 6, 1983 (Fig. 3)
≡ *Cercospora cladosporioides* Saccardo, *Michelia* 2: 556, 1882

on **Olea europaea* L. (Oleaceae) Auckland, Sandringham, 9.IX.1987, P. R. Johnston (PDD 48034). Waikato, Hamilton, University grounds, 7.VII.1984, J. M. Dingley (PDD 46175). Hawkes Bay, Napier, 24.VIII.1970, I. J. Ryan (PDD 28571).

Black leaf mould, fruit spot. Mould growth initially circular, but coalescing and becoming irregular, to cover lower surface of leaf. On fruit, spots purple (Hansen & Rawlins 1944), becoming greyish-black with onset of sporulation, circular or oval, up to 1 cm diam. On leaves, primary mycelium internal, pale, branched, septate, 2–3 µm wide, sometimes forming swollen cells up to 6 µm wide. Secondary mycelium external, repent, pale-brown, branched, septate, 2–5 µm wide, giving rise to conidiophores. Conidiophores pale-brown, smooth, straight or flexuous, up to 150 µm long × 3.5–5 µm wide, sometimes branched. On fruit, conidiophores fasciculate, up to 30 µm long, arising from brown stromata which are up to 200 µm wide × 150 µm deep. Conidia pale-brown, smooth, subcylindric, straight or curved, rounded at the apex, basal cell sometimes slightly tapered to truncate or slightly convex hilum, often with a small, inconspicuous marginal frill, 1–8 septate, (29–)65–85(–90) × (3–)3.5–5(–5.5) µm.

This disease is known from southern Europe, Algeria, and California. Hansen & Rawlins (1944) described fruit symptoms, and found that infected fruit were unsuitable for pickling as green olives. Leaf mould may cause early drop of infected leaves.

Phaeoramularia eupatorii-odorati (Yen) Liu et Guo, *Acta phytopath. sin. 12(4)*: 7, 1982 (Fig. 4).
≡ *Cercospora eupatorii-odorati* Yen, *Bull. Soc. mycol. Fr. 84*: 10–11, 1968

on **Ageratina adenophora* (Spreng.) King et Robinson (Asteraceae) Northland, Whangaroa, Mar. 1963, S. Davison (PDD 21169); Opito Bay, 29.VI.1973, J. M. Dingley (PDD 30893). Coromandel, Port Charles, Sandy Bay, 31.XII.1962, S. Davison (PDD 20834); Port Charles, 18.IX.1988, P. R. Johnston (PDD 54600); Whangapoua Saddle, 5.IX.1963, J. M. Dingley (PDD 21812). Auckland, Orere, 6.I.1966, R. F. R. McNabb (PDD 24849).

Dingley (1965) recorded and described *Cercospora eupatorii* Peck on *Eupatorium adenophorum* Spreng.

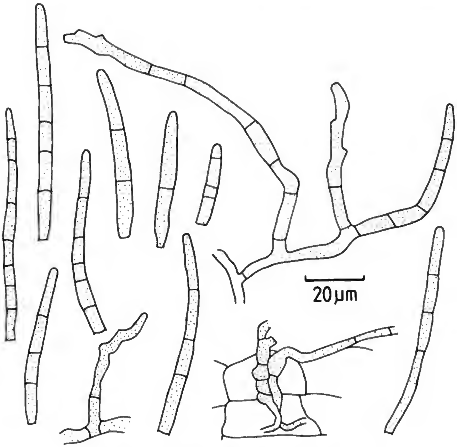


Fig. 3 Conidia and conidiophores of *Mycocentrospora cladosporioides* (PDD 48034). Specimen mounted in hydrous lactophenol.

(≡ *Ageratina adenophora*). An examination of the three specimens cited by Dingley, and of more recent collections, has shown that the conidia are often catenate, and that the fungus is a species of *Phaeoramularia*. The conidia of PDD 20834, on which Dingley's description was probably based, are obclavate and not in chains (Fig. 4). However, all other collections have conidia which are mainly cylindric and catenate (Fig. 4). Dingley suggested that the fungus was introduced to New Zealand on a trypetid stem gall fly (*Procecidochares utilis* Stone), an insect introduced from Queensland for the control of *Eupatorium*. Simmonds (1966) recorded *C. eupatorii* on *E. adenophorum* in Queensland. Two specimens (PDD 23521 and 23522) collected in Queensland in July 1964 were examined and found to be *P. eupatorii-odorati*.

Phyllachora bulbosa Parbery, *Aust. J. Bot. 15*: 299-300, 1967 (Fig. 5A)

on *Zoysia pauciflora* Mez (Poaceae) Bay of Plenty, Whale Island, 4.I.1986, R. E. Beever (PDD 49700).

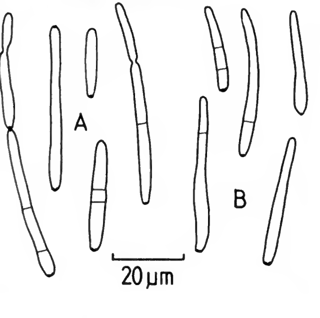
on *Zoysia planifolia* Zotov (Poaceae) Northland, Pukenui, 3.I.1954, L. B. Moore (PDD 40425 ex CHR 83553); Houhora, 21.XII.1971, A. E. Esler (PDD 41197). Auckland, Kaipara Heads, Pouto, Dec 1904, D. Petrie (PDD 40417 ex CHR 5854).

Tar spot. Colonies black, discrete, raised, amphigenous and on culm below flower head, up to 1 × 0.25 mm. Asci cylindrical 70–100 × 9–12(–15) μm; paraphyses sinuous, up to 130 μm long × 1–2 μm wide. Ascospores monoseriate, ellipsoid, subglobose, or oval, 12–16(–17) × (6.5–)7–9 μm. Spermogonia present within some colonies. Spermatiophores 11–12 × 2.5–3 μm. Spermatia filiform 8–15 × 0.5–0.75 μm.

The New Zealand collections of this fungus differ slightly from the type, and only specimen of *Phyllachora bulbosa* described from Australia. The Australian fungus has narrower ascospores (13–17 × 6.5–7.5 μm), and longer paraphyses (twice as long as asci) (Parbery 1967). Spermatiophores of the New Zealand collections do not appear to have a swollen, bulbous base as found in the Australian specimen.

Phyllachora setariicola Spegazzini, *An. Soc. cient. argent. 19*: 246, 1885 (Fig. 5B)

on *Oplismenus hirtellus* (L.) Beauv. (Poaceae) Northland, Opito Bay, 29.VI.1973, J. M. Dingley (PDD 30910). Auckland, Piha, Sep 1950, J. M. Dingley (PDD 15930); Titirangi, Clark's Bush, 3.V.1979, E. H. C. McKenzie (PDD 3945



ig. 4 Conidia of *Phaeoramularia eupatorii-odorati* , PDD 54600; B, PDD 20834). Specimens mounted in ydrous lactophenol.

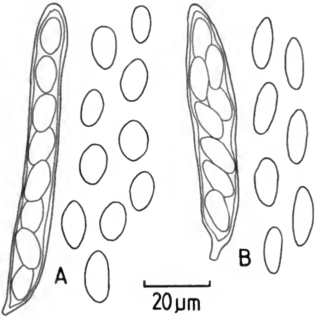


Fig. 5 Ascus and ascospores of A, *Phyllachora bulbosa* (PDD 49700) and B, *P. setariicola* (PDD 39453). Specimens mounted in hydrous lactophenol.

oromandel, Little Barrier Island, 5.IV.1988, E. H. . McKenzie (PDD 48737). Waikato, Te Kauri eserve, 20.V.1988, E. H. C. McKenzie (PDD 8860).

ar spot. Colonies black, discrete or confluent, often near, raised, amphigenous and on leaf sheath, up to .25 × 0.1 mm. Asci cylindrical or saccate, 60–90 × 2–16 μm. Ascospores monoseriate or biseriate, llipsoid or oval, 15–17(–19) × 6–7(–8) μm.

n New Zealand, this common species was recorded s *Phyllachora punctum* (Schwein.) Orton on *plismenus* (Dingley 1969). However, *P. punctum* as smaller (9–13 × 4–5.5 μm), strictly monostichous scospores (Parbery 1967). *P. setariicola* is known om Central and South America, Africa, Japan, outh-East Asia, Australia, and many Pacific island ations. It occurs on many grasses within the ubfamily Panicoideae.

uccinia festucae Plowright, *Grevillea 21*: 109, 893

n **Festuca rubra* L. (Poaceae) Mid Canterbury, raigieburn State Forest Park, 24.II.1988, E. H. C. cKenzie (PDD 48435) II; Lake Sarah, 25.II.1988, . H. C. McKenzie (PDD 48449) II.

eaf rust. Uredinia on adaxial leaf surface, ulverulent, golden-yellow. Urediniospores 25–)27–30(–33) × (21–)23–26(–28) μm, globoid r broadly ellipsoid, wall 1.5–2(–2.5) μm thick, pale golden, echinulate, germ pores 6–8, scattered; pedicels sometimes persisting.

This rust which occurs on *Festuca* spp. in North America, Europe, India, and Korea, was figured and described by Cummins (1971).

Tolyposporium rodwayi McAlpine, *The Smuts of Australia*: 189, 1910 (Fig. 6)

on *Lepidosperma laterale* R. Br. (Cyperaceae) Auckland, Piha Valley, 7.V.1978, A. E. Esler (PDD 55626).

Smut. Sori in the inflorescence, forming granular, black spore ball masses. Spore balls up to 90 × 60 μm, often about 50 × 40 μm, subglobose, polyhedral, oblong, or irregular, opaque, composed of many, radially arranged, firmly agglutinated spores. Spores in surface view 8–11 × 6–10 μm, polygonal, in median view 12–25 × 7–10 μm, elongate or wedge-shaped, light- to dark-brown, wall smooth on contact sides, densely verruculose on free surface.

Tolyposporium rodwayi was described from Tasmania on *Lepidosperma laterale*. In Australia, it also occurs on *L. elatius* Labill.

ADDITIONAL HOST RECORDS

The following 31 fungi have been previously recorded on other hosts in New Zealand, details of which are given by Pennycook (1989).

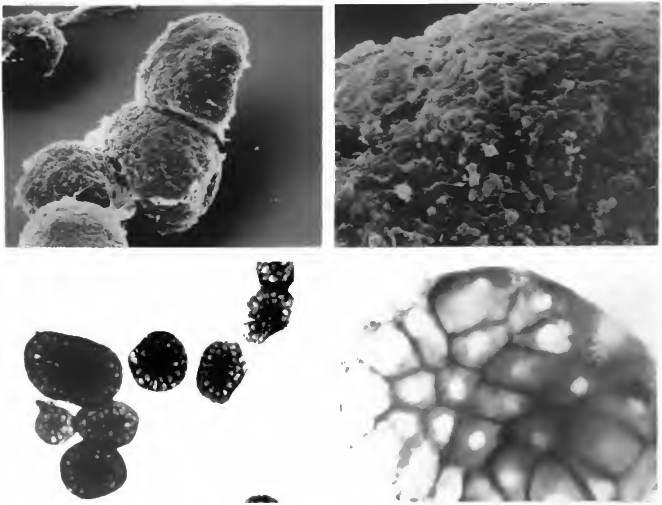


Fig. 6 Spore balls of *Tolyposporium rodwayi* (PDD 55626). Scanning electron microscope specimens upper. Light microscope specimens (lower) mounted in hydrous lactophenol. Bar = 10 µm.

Albugo candida (Pers.) O. Kuntze

on *Lepidium banksii* Kirk (Brassicaceae) Nelson, near Nelson, 9.XII.1988, S. P. Courtney (PDD 55106).

Alternaria dichondrae Gambogi, Vannacci et Triolo

on **Dichondra micrantha* Urb. (Convolvulaceae) Auckland, Mt Albert Research Centre, DSIR, 1988, A. E. Esler (PDD 55787).

Botryotinia draytonii (Buddin et Wakef.) Seaver
anamorph: *Botrytis gladiolorum* Timmermans

on **Ixia polystachya* L. (Iridaceae) Auckland, Waikumete Cemetery, 17.X.1988, A. E. Esler (PDD 54817)

on **Ixia viridiflora* Lam. (Iridaceae) Wellington, Karori, 12.X.1963 (PDD 21[illegible]).

Botrytis cinerea Pers.

on **Hibiscus diversifolius* Jacq. (Malvaceae) Auckland, Royal Oak, 28.VI.1987, R. E. Beever (PDD 53661).

Cercospora chenopodii Fres.

on **Atriplex patula* L. (Chenopodiaceae) Auckland, Weymouth, 12.IV.1976, J. M. Dingley (PDD 34775). Coromandel, Little Barrier Island, 6.IV.1988, E. H. C. McKenzie (PDD 55788).

Cercosporidium graminis (Fuckel) Deighton

on *Hierochloe redolens* (Solander ex Vahl) Roem. et Schult. (Poaceae) North Canterbury, Arthur's Pass, Greyney's shelter, 9.II.1989, E. H. C. McKenzie (PDD 55636).

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lletotrichum graminicola (Ces.) Wilson

**Alopecurus geniculatus* L. (Poaceae) Westland, arihari, 11.II.1989, E. H. C. McKenzie (PDD 005)

**Echinochloa cruspavonis* (H.B.K.) Schult. oaceae) Auckland, Mt Albert, 22.IV.1988, E. H. McKenzie (PDD 48694).

iplocarpon rosae Wolf anamorph: *Marssonina sae* (Lib.) Died.

**Rosa rubiginosa* L. (Rosaceae) Mid Canterbury, ass, 19.XI.1988, E. H. C. McKenzie (PDD 55150).

ntyloma dactylidis (Pass.) Cif.

**Alopecurus geniculatus* L. (Poaceae) Westland, arihari, 11.II.1989, E. H. C. McKenzie (PDD 5770)

n *Elymus narduroides* (Turcz.) Löve et Connor oaceae) Mid Canterbury, near Springfield, junction owai and Rubicon Rivers, Mar. 1948, H. Talbot DD 55601 ex CHR 301427).

uehneola uredinis (Link) Arthur

n *Rubus schmidelioides* A. Cunn. (Rosaceae) Mid anterbury, Cass, 19.XI.1988, E. H. C. McKenzie PDD 55111) II.

ycosphaerella spissa H. Sydow

n *Coprosma serrulata* Hook. f. ex Buchan. Rubiaceae) North Canterbury, Arthur's Pass, 0.XI.1988, E. H. C. McKenzie (PDD 55112).

hragmidium constrictosporum Laundon

n *Acaena anserinifolia* (J.R. et G. Forst.) Armstr. Rosaceae) Mid Canterbury, Cass, 22.II.1988, E. H. . McKenzie (PDD 48669) II, III.

hysoderma potteri (Bartlett) Karling

n **Lotus pedunculatus* Cav. (Fabaceae) Auckland, Vaitakere Ranges, Sharp's Bush, 5.XI.1965, J. S. arling (PDD 24811).

arling (1966) recorded *P. potteri* on *L. corniculatus*. owever, according to Webb et al. (1988) *L. orniculatus* has been frequently recorded in error as aturalised in New Zealand. The host for the single pecimen in PDD has been redetermined as *L. edunculatus*. Karling also recorded a second locality (Kauaeranga Valley) for this fungus. Unfortunately, there is no specimen in PDD to substantiate the latter record, or to determine the host species.

Pseudocercospora lonicericola (Yamam.) Deighton

on **Lonicera japonica* Thunb. (Caprifoliaceae) Auckland, Waitakere Ranges, Cascades Kauri Park, 17.III.1956, J. M. Dingley (PDD 15983); Walker's Bush, 4.VIII.1982, E. H. C. McKenzie (PDD 43117).

Pseudocercospora myrticola (Speg.) Deighton

on *Metrosideros excelsa* Sol. ex Gaertn. (Myrtaceae) Taranaki, Waitara, Aug 1983, F. Jury (PDD 44470).

Puccinia coronata Corda

on **Alopecurus geniculatus* L. (Poaceae) Westland, Harihari, 11.II.1989, E. H. C. McKenzie (PDD 55751) II.

Puccinia graminis Pers.

on **Vulpia bromoides* (L.) S. F. Gray (Poaceae) Mid Canterbury, Little Lake Lyndon, 26.II.1988, E. H. C. McKenzie (PDD 48678) II.

Puccinia hordei Otth

on **Vulpia myuros* (L.) C.C. Gmel. (Poaceae) Mid Canterbury, Christchurch, Riccarton, 11.XII.1987, A. J. Healy (PDD 48421) II, III.

Puccinia maurea G.H. Cunn.

on *Carex echinata* Murray (Cyperaceae) Marlborough, Lewis Pass, St James Walkway, 8.II.1989, E. H. C. McKenzie (PDD 55616) II.

Puccinia oxalidis Diet. et Ellis

on **Oxalis brasiliensis* Lodd. ex Westc. et Knowles (Oxalidaceae) Mid Canterbury, Christchurch, Riccarton, 28.VIII.1989, A. J. Healy (PDD 56209) II.

Although growing in close proximity to other heavily infected *Oxalis* spp., *O. brasiliensis* had only slight infection on a few leaves, comparable to that previously observed on *O. pes-caprae* L. (A. J. Healy pers. comm.).

Puccinia recondita Roberge ex Desm.

on *Elymus narduroides* (Turcz.) Löve et Connor (Poaceae) Mid Canterbury, near Springfield, junction Kowai and Rubicon Rivers, Mar. 1948, H. Talbot (PDD 55602 ex CHR 301427) II.

Puccinia tenuispora McAlp.

on *Luzula crinita* Hook. f. var. *petriana* (Buch.) Edgar (Juncaceae) Mid Canterbury, Temple Basin, 25.II.1988, E. H. C. McKenzie (PDD 48681) II.

Pucciniastrum pustulatum Diet.

on **Fuchsia magellanica* Lam. var. *macrostema* (Ruiz Lopez et Pavon) Munz (Onagraceae) Waikato, Hamilton, Sillury Street Gardens, 22.I.1989, E. H. C. McKenzie (PDD 55116) II

on **Zauschneria californica* Presl (Onagraceae) Mid Canterbury, Lincoln, 10.III.1987, F. C. Allen (PDD 53595) II.

Ramularia sphaeroidea Sacc.

on **Lotus pedunculatus* Cav. (Fabaceae) Northland, Coopers Beach, 21.IX.1977, E. H. C. McKenzie (PDD 37242). Auckland, Oratia, 15.XI.1956, S. D. Brook (PDD 16832). Taranaki, Mt Egmont, 29.III.1951, J. M. Dingley (PDD 10623). Coromandel, Little Barrier Island, 6.IV.1988, E. H. C. McKenzie (PDD 48721). Wellington, Palmerston North, Massey, 23.X.1963, G. C. M. Latch (PDD 33754). Westland, Fox River, 1.II.1966, H. C. Smith (PDD 31777).

Septoria fuchsiicola P. Sydow

on *Fuchsia excorticata* (J.R. et G. Forst.) Linne f. (Onagraceae) Taupo, Pureora Forest, Kakaho picnic area, 19.II.1989, R. E. Beever (PDD 55796). Marlborough, Lewis Pass, Waterfall track, 8.II.1989, E. H. C. McKenzie (PDD 55653).

Septoria rubi Westend.

on **Rubus squarrosus* Fritsch (Rosaceae) Mid Canterbury, Cass, 22.II.1988, E. H. C. McKenzie (PDD 48687).

Sorosporium solidum (Berk.) McAlp.

on *Schoenus apogon* Roem. et Schult. var. *apogon* (Cyperaceae) Auckland, Waikumete Cemetery, 1.IX.1976, S. Bowman & W. S. Versluys (PDD 55625); Waikumete Cemetery 26.X.1989, E. H. C. McKenzie (PDD 56324).

Uredo puawhananga Baylis

on *Clematis cunninghamii* Turcz. (Ranunculaceae) Northland, Whangaruru North Head, 28.I.1988, P. R. Johnston (PDD 48393) II; Waiotemara Bush Walk, 28.I.1988, P. R. Johnston (PDD 48394) II.

Uredo spyridii Cooke et Massee

on **Pomaderris aspera* DC. (Rhamnaceae) Auckland, Epsom, 31.V.1986, R. E. & J. E. Beever (PDD 52052) II.

Uromyces otakou G.H. Cunn.

on *Poa imbecilla* Spreng. (Poaceae) Mid Canterbury, Cass, Andrews Stream, 19.XI.1988, E. H. C. McKenzie (PDD 54919) II.

Uromyces rumicis (Schumacher) Winter

on **Rumex conglomeratus* Murray (Polygonaceae) Coromandel, Little Barrier Island, 7.IV.1988, E. H. C. McKenzie (PDD 48869) II, III.

RECORDS TO BE DELETED

Cercospora eupatorii Peck

on **Eupatorium adenophorum* Spreng. (Dingley 1965)

This pathogen has been redetermined as *Phaeoramularia eupatorii-odorati* (q.v.).

Phyllachora punctum (Schwein.) Orton

on *Oplismenus hirtellus* (L.) Beauv. (Dingley 1969)

This pathogen has been redetermined as *Phyllachora setariicola* (q.v.).

Ramularia sphaeroidea Sacc.

on **Lotus corniculatus* L. (Brien & Dingley 1953)

Brien & Dingley (1953) recorded this fungus, as *Ovularia exigua* (W. G. Smith) Sacc., on *L. corniculatus*. According to Webb et al. (1988) *L. corniculatus* has been frequently recorded in error as naturalised in New Zealand. Examination of all *R. sphaeroidea* specimens held in PDD, including a Dingley specimen collected prior to 1953, has revealed that the host plant is *L. pedunculatus* Cav.

Ustilago agropyri McAlp.

on **Anthoxanthum odoratum* L. (Cunningham 1924)

This indigenous smut, originally described from Australia, is common and widespread in New Zealand on *Rytidosperma* spp. Cunningham (1924) recorded it, as *Ustilago readeri* Sydow, on *Anthoxanthum odoratum*. Its occurrence on both Australasian species of *Rytidosperma* and on the northern hemisphere *A. odoratum* appeared unlikely. Following examination of the specimen on *A. odoratum* (PDD 1248), the host was redetermined by Mr A. E. Esler as *Rytidosperma* sp.

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ook review

aboratory guide for identification of plant athogenic bacteria. 2nd edition. Edited by N. W. chaad. Published in 1988 by American hytopathological Society Press, St Paul, Minnesota. 8 pages, plus Contents, plates, etc; soft covered. BN 0–89054–078–0. Price (including postage): nited States US$28.00; elsewhere US$34.00.

his second edition is divided into four parts: entification schemes, Gram-negative bacteria, ram-positive bacteria, and cell wall-free bacteria. ach of the three parts reporting on a bacterial group further divided into chapters on the bacterial enera containing plant pathogens, written by authors ith expertise in the genera treated. Each chapter utinely contains an introductory section, followed y sections on isolation techniques, differentials of ommonly isolated species, diagnostic media and sts, pathogenicity tests, literature cited, and an dex of chemicals used. The chemicals index is a seful innovation emphasising, as it does, the need prepare media precisely to obtain satisfactory reults. Sections with relevance to particular bacterial roups—opine catabolism for *Agrobacterium* sp., anthomonadin production for *Xanthomonas* spp., erological methods for cell wall-free bacteria, etc.—re included in appropriate chapters.

In this edition, there is a new chapter on *Xylella* nd other fastidious bacteria, the part on Gram-ositive bacteria associated with plants has been xpanded to include *Bacillus*, *Clostridium*, and *treptomyces*, the part on cell wall-free bacteria has een significantly updated, and the many typographic rrors which marred the first edition have been orrected. This edition uses scroll-binding rather han the earlier cumbersome ring-binding, underining a decision to produce individual editions ather than to arrange for up-dating by page insertion. There is a useful collection of colour plates illustrating growth on selected media, and plant reactions.

The chapters on pathogenic genera give information, largely from the direct experience of the individual authors, on media and methods for laboratory and glasshouse experiments by which strains can be identified. The calibre of individual chapters overall is high, reflecting the expertise of the contributors. Specific information is necessarily variable in quality, depending upon the extent to which the organisms have been studied.

This handbook would have been better served by a clear exposition in its introduction of the audiences at which it is aimed. The introductory part, "Identification Schemes", indicates a procedure by which bacterial strains from primary isolations from plant material can be keyed to genus, and the routine reporting of procedures for the isolation of pathogens from plant tissue indicates the intention to provide a diagnostic guide for diagnosticians. However, although the handbook is a useful compilation of laboratory identification methods and recipes for media which are otherwise scattered in other publications, and is well suited as a compendium for research workers needing methods to screen for a specific pathogen, those seeking to use this handbook primarily for diagnostic purposes may be disappointed by the presentation. Diagnosis of plant pathogens aims at the reliable identification of a causal organism in the most rapid way. The handbook specifically offers details for the identification of putative plant pathogenic bacterial strains but does not include or make reference to a host-pathogen index which, for practical purposes, is the starting point of identification for routine diagnostic work. Proficiency in isolation, the confident maintenance of pure subcultures, up-to-date host-pathogen lists from the international literature, the ability to implement those tests which most rapidly discriminate pathogens from the host in question, and the capacity to prove virulence of putative pathogens when necessary, are the cornerstones of diagnostics of plant pathogenic bacteria.

Considering the number of pathogens treated in detail (more than 100 pathogens in about 15 taxonomic groups), the diagnostic purposes of the handbook would have been well served by an expanded, revised version of the flow guide shown in the introductory chapter, to give as coherent as possible a picture of the procedures for the diagnosis of plant pathogenic bacteria, however limited this might be. The incompleteness of keys for identification of pathogens would be a graphic demonstration of the limited state of present knowledge.

As in the first edition, considerable emphasis is laid on the use of selective media for the identification of particular pathogens. Perhaps there should have been a caveat on the use of some of these media, which may not be specific to the reported pathogen, and those media whose plating efficiency to a range of strains of the pathogen of interest may be uncertain. Some media given for pathovars of *Pseudomonas syringae* (e.g., medium MSP) and *X. campestris* (e.g., medium D–5) are not usually specific for particular pathovars as implied, but can be used for selecting isolates of the species from plants or plant environments, thus allowing the identity of the pathogen to be inferred, or proved by other means. Although specific selective media are potentially useful in studies where a particular pathogen is under routine study, and where the medium has been confirmed as specific within the environment of the investigation it would be interesting to know if selective media are widely used in diagnostic laboratories. The preparation of these often complex and labile media sets a practical limit on their use for routine purposes that is not apparent from a reading of this text, and may not be justified in terms of worthwhile improvement in confident identification of a pathogen.

It is also interesting to speculate about the extents to which this handbook expresses academic, rather than practical, identification proposals. The Gram reaction is very important in the formal confirmation of the identity of a bacterium, but who, one wonders, follows the injunction to use this test as a first step in the protocol for identification of strains of plant pathogenic bacteria?

This guide to identification would be assisted by an account of the relationship of identification to naming and to classification of plant pathogenic bacteria. This is particularly appropriate in the context of plant pathogenic bacteria which are commonly classified and named, and to a lesser extent identified, according to their pathogenic characters. Notwithstanding the primacy of pathogenicity in nomenclature, for many host-pathogen combinations, reliable methods for proving pathogenicity have not been published. This is one of the important lacunae in our elementary knowledge of plant pathogenic bacteria.

Molecular biological methods receive little mention. Clearly these methods will transform bacterial identification and it is surprising that their use is not anticipated here. A section on plasmid screening is included for *Agrobacterium*, but other methods reported in identification studies before 1987, such as colony hybridisation using genomic DNA probes (then routine in some laboratories) and genomic finger-printing, are not mentioned.

This handbook is a useful asset in plant bacteriology laboratories engaged in the identification of specific pathogens in research. Together with **Methods for the diagnosis of bacterial diseases of plants** (R. A. Lellicott and D. E. Stead, Blackwell, 1987), **Guide to plant pathogenic bacteria** (J. F. Bradbury, CAB International Mycological Institute, 1986), and **Plant bacterial diseases—a diagnostic guide** (edited by P. C. Fahy and G. J. Persley, Academic Press, 1983), it offers the best available collection of information for those involved in the identification and diagnosis of plant pathogenic bacteria.

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